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KNOB POSITIONS ON CORN CHROMOSOMES[1]

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INTRODUCTION

Chromosome knobs are enlargements on the chromosomes of corn (*Zea mays* L.) and its near relatives, visible on the threads at prophase of the first meiotic division. These enlargements stain deeply with carmine and vary in size from swellings but slightly more prominent than the adjacent chromomeres to those that equal the volume of a whole chromosome at anaphase.

McClintock[2] was the first to report knobs on the midprophase chromosomes of corn. Since this first report, several investigators have found that knobs on the chromosomes of corn are seemingly a constant feature and aid materially in the identification of many of the 10 chromosomes.

The writer was impressed by the fact that the location of the various knobs was not at random and that certain knobs are present in nearly all plants examined, whereas others are present in a very few plants. The location of the knobs and the unequal frequency with which they occur on the 20 arms of the chromosomes suggested the possibility that their frequency might be related to their position.

MATERIAL AND METHODS

The data for both knob frequency and knob position were obtained from camera lucida drawings, made at a magnification of $\times$ 3,000, of chromosomes in pollen mother cells of the 33 Indian varieties of corn from the United States that were described in a previous publication,[3] and a similar collection of 41 varieties from isolated regions of Mexico.

The usual carmine-smear technique for preparing pollen mother cells was used. The cells were pressed to flatten the chromosome threads as much as possible without rupturing the cells. All measurements are given in microns, and the slight error, due to the fact that chromosomes were not always brought into one plane throughout their whole length, has been neglected because corrections are tedious and would seem to affect the results very little.

LENGTH OF THE CHROMOSOMES

The length of corn chromosomes is found to vary in pollen mother cells, depending on the stage of development. Measurements in the

[1] Received for publication July 24, 1939.

[2] MCCLINTOCK, BARBARA. A CYTOLOGICAL DEMONSTRATION OF THE LOCATION OF AN INTERCHANGE BETWEEN TWO NONHOMOLOGOUS CHROMOSOMES OF ZEA MAYS. Natl. Acad. Sci. Proc. 16: 791-796, illus. 1930.

[3] LONGLEY, A. E. CHROMOSOMES OF MAIZE FROM NORTH AMERICAN INDIANS. Jour. Agr. Res. 56: 177-195, illus. 1938.

present study have been restricted to those figures in which the chromosome threads are much extended. It has been found that chromosomes contracted to less than one-half their length at the most extended stage are not satisfactory for observing the various characteristic markings on the threads. The smaller knobs, for example, in much-contracted threads are not sharply differentiated from the normal thickening of the thread which results from the contraction.

The measurements of all chromosomes in 28 different cells from 14 Indian varieties are given in table 1; only two of these measurements are from the same plant. This small group of measurements represents the general results of measuring the length of corn chromosomes and clearly demonstrates that chromosome identification must rely on features other than relative length, such as the fiber-attachment position, the presence of knobs, and other less prominent characteristics.

TABLE 1.—*Length of midprophase chromosomes of corn from 28 pollen mother cells*

Cell No.	Length of chromosome No. —									
	I	II	III	IV	V	VI	VII	VIII	IX	X
	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ
1	80.00	80.83	79.17	73.33	72.50	65.00	54.17	52.50	50.00	42.07
2	93.33	68.33	75.83	67.50	56.67	50.00	45.83	52.50	50.00	44.10
3	98.33	76.67	77.50	60.83	75.00	53.33	60.83	59.17	62.50	46.62
4	76.67	62.50	57.50	60.00	61.67	49.17	54.17	36.67	46.67	41.18
5	80.83	78.33	73.33	73.33	56.67	47.50	49.17	59.17	50.83	43.33
6	100.67	77.50	95.83	63.33	94.17	55.83	64.17	45.00	72.50	52.75
7	85.83	70.00	69.17	54.17	58.33	47.50	49.17	43.33	42.50	35.16
8	84.17	64.17	70.83	66.67	70.83	48.33	47.50	50.83	52.50	46.29
9	83.33	79.17	65.83	66.67	59.17	39.17	39.17	49.17	38.33	31.77
10	51.67	35.00	34.17	31.67	33.33	30.00	25.00	28.33	39.17	32.02
11	50.83	45.83	44.17	44.17	38.33	36.67	33.33	36.67	30.83	27.98
12	87.50	82.50	60.00	67.50	56.67	47.50	42.50	38.33	51.67	40.41
13	83.33	63.33	63.33	60.00	65.83	38.33	37.50	42.50	42.50	36.85
14	72.50	61.67	62.50	55.00	61.67	37.50	45.83	44.17	40.83	33.97
15	54.17	41.67	40.00	38.33	40.83	34.17	30.00	30.00	29.17	27.73
16	84.17	64.17	52.50	66.67	53.33	35.00	50.00	45.83	36.67	35.13
17	72.50	61.67	53.33	49.17	52.50	38.33	42.50	40.83	40.00	36.20
18	140.00	93.33	95.83	87.50	89.17	82.50	81.67	77.50	56.67	46.16
19	87.50	66.67	67.50	64.17	66.67	54.17	45.00	49.17	39.17	32.79
20	55.00	42.50	40.83	40.83	37.50	31.67	32.50	33.33	30.83	30.01
21	54.17	37.50	33.33	32.50	34.17	29.17	29.17	30.00	30.83	26.45
22	57.50	35.83	42.50	35.83	35.83	39.17	26.67	26.67	24.17	25.00
23[1]	67.50	57.50	52.50	50.00	52.50	38.33	36.67	39.17	39.17	33.23
24[1]	70.00	60.83	53.33	41.67	44.17	34.17	31.67	35.83	31.67	27.53
25	92.50	76.67	71.67	58.33	57.50	49.17	44.17	46.67	52.50	44.62
26	69.17	52.50	62.50	52.50	45.00	36.67	35.83	40.00	28.33	25.74
27	63.33	44.17	43.33	38.33	40.00	30.00	32.50	39.17	31.67	28.96
28	90.00	110.00	99.17	73.33	84.17	70.83	60.83	65.83	63.33	51.54
Mean	78.09	63.96	62.05	56.19	56.93	44.61	43.84	44.23	43.04	36.63

[1] Cells 23 and 24 were from the same plant.

Table 2 presents separately the mean lengths of the 10 chromosomes of two corn groups and then combines these groups—the United States Indian varieties and the Mexican varieties—to give a general mean for each chromosome length. It is clear from the means of tables 1 and 2 that there is a general reduction in the length of the chromosomes from I to X but that chromosomes V and VIII are on an average slightly longer than chromosomes IV and VII, respectively.

The length of a chromosome seems never to be fixed. In the first meiotic prophase there is a gradual shortening in length until the metaphase is reached. Consequently, the measurements reported in tables

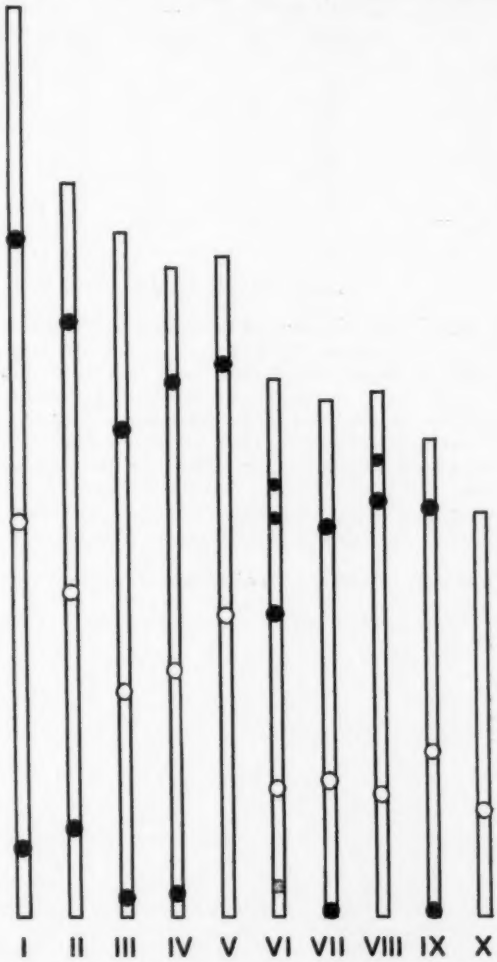

FIGURE 1.—Diagram of the 10 chromosomes of corn, showing knobs (black) and fiber attachments (circles). × 1,500.

1 and 2 are given to show relative lengths only at that phase when knobs are most prominent and their positions most readily fixed. Figure 1 shows diagrammatically the relative lengths, as given in the last column of table 2, of all 10 chromosomes.

TABLE 2.—*Mean length of midprophase corn chromosomes*

Chromosome No.	United States Indian varieties		Mexican varieties		Combined United States Indian and Mexican varieties	
	Measurements	Mean length	Measurements	Mean length	Measurements	Mean length
	Number	μ	*Number*	μ	*Number*	μ
I	250	82.83	93	81.22	343	82.40
II	218	66.32	88	66.95	306	66.50
III	262	61.62	92	63.09	354	62.00
IV	240	58.72	102	58.91	342	58.78
V	240	60.08	80	59.05	320	59.82
VI	270	49.33	107	47.23	377	48.73
VII	261	46.71	106	46.96	367	46.78
VIII	258	47.21	103	48.16	361	47.48
IX	313	42.79	103	44.59	416	43.24
X	279	37.07	112	36.59	391	36.93

LENGTH OF ARMS

The fiber attachment divides each chromosome into two arms. In this study of knob position, each arm is considered independently of the length of the whole chromosome and in consequence the mean lengths of the 20 arms of the 10 chromosomes, given in table 3, are more relevant to the problem under discussion than are the chromosome lengths given in tables 1 and 2. In table 3 the chromosome arms are arranged in order of length, beginning with the shortest and ending with the longest. The number of measurements is the same as in table 2 in each case. Figure 2 shows diagrammatically the different lengths of the 20 arms, as given in the last column of table 3.

TABLE 3.—*Mean length of arms of midprophase chromosomes of corn*

Chromosome		Length of arm of—			Chromosome		Length of arm of—		
No.	Arm	United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	No.	Arm	United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
		μ	μ	μ			μ	μ	μ
X	Short	9.76	9.93	9.81	II	Short	29.31	30.01	29.51
VIII	do	11.09	11.69	11.26	V	Long	32.78	31.44	32.45
VI	do	11.68	12.50	11.91	VII	do	34.18	34.72	34.34
VII	do	12.53	12.23	12.44	I	Short	36.15	35.14	35.87
IX	do	15.01	15.81	15.21	VIII	Long	36.12	36.47	36.22
III	do	20.46	20.67	20.51	IV	do	36.03	36.96	36.31
IV	do	22.69	21.95	22.47	VI	do	37.65	34.73	36.82
X	Long	27.31	26.66	27.12	II	do	37.01	36.88	36.97
V	Short	27.29	27.61	27.37	III	do	41.16	42.42	41.49
IX	Long	27.78	28.77	28.03	I	do	46.69	46.09	46.52

KNOB POSITIONS

Knobs have been found on all but 5 of the 20 arms of the 10 chromosomes, disregarding the knoblike enlargement comprising the nucleolus "organizer" in the short arm of chromosome VI. Thirteen arms apparently have only 1 knob each, while 1 of the 2 remaining arms may have 2 knobs and the other may have 3.

Two convenient ways of expressing the position of a knob are as its distance from the end of the arm and as its distance from the fiber attachment. Table 4 shows the mean position of all knobs expressed in these two ways, and figure 2 shows diagrammatically the mean positions of all 18 knobs.

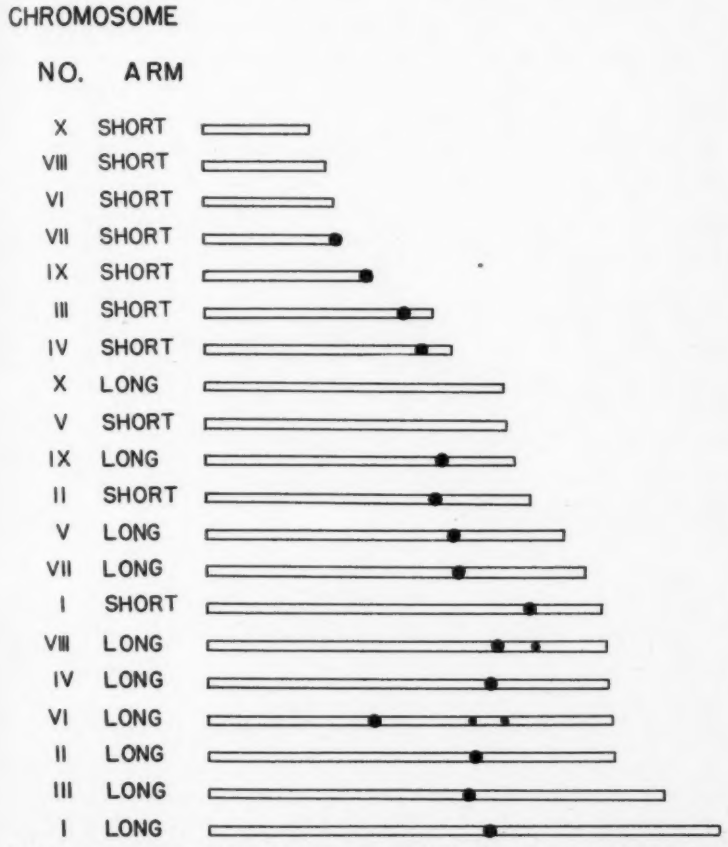

FIGURE 2.—Diagram of the 20 chromosome arms of corn, arranged in order of their length and showing knobs (black), the left end of each arm being the fiber-attachment point. × 1,500.

Although these 18 knobs differ widely in the frequency of their occurrence, each deviates very little from its mean position; therefore, all students of corn chromosomes think of each knob as being constant in position. The fact that a knob is in the same position on the chromosome, even in plants coming from widely separated localities, suggested that there are 18 points on the 20 chromosome arms having the ability to form a knob under certain conditions. These points doubtless exist on the chromosome threads of all corn plants but are visible only when knobs are formed. Consequently, the data have

been examined to determine whether the frequency with which a knob is found depends on its position on the chromosome thread.

TABLE 4.—*Mean distance of knobs from fiber attachment and from end of chromosome arms*

Knob location on chromosome—		United States Indian varieties		Mexican varieties		Combined United States Indian and Mexican varieties	
		Distance of knob from—		Distance of knob from—		Distance of knob from—	
No.	Arm	End	Fiber attachment	End	Fiber attachment	End	Fiber attachment
		μ	μ	μ	μ	μ	μ
VII	Short	0.00	12.52	0.00	12.23	0.00	12.44
IX	do	.00	15.01	.00	15.81	.00	15.21
III	do	2.25	18.21	1.50	19.17	2.00	18.51
IV	do	3.00	19.69	2.17	19.78	2.45	20.01
IX	Long	5.64	22.15	7.63	21.11	6.25	21.78
II	Short	8.75	20.56	8.16	21.86	8.53	20.99
V	Long	9.83	22.95	9.56	21.88	9.75	22.70
VII	do	11.35	22.83	11.39	23.33	11.37	22.97
I	Short	6.24	29.91	6.71	28.43	6.46	29.41
VIIIa [1]	Long	5.36	30.76	6.67	29.81	6.24	29.98
VIIIb [2]	do	9.46	26.66	10.50	25.98	9.79	26.43
IV	do	10.33	25.70	10.36	26.60	10.35	25.96
VIa [1]	do	9.89	27.77	9.08	25.65	9.64	27.18
VIb [2]	do	12.77	24.89	12.38	22.35	12.65	24.17
VIc [3]	do	21.34	16.31	20.86	13.87	21.29	15.53
II	do	13.03	23.97	11.87	25.01	12.63	24.35
III	do	17.28	23.89	18.65	23.77	17.84	23.65
I	do	19.88	26.81	23.33	22.76	20.91	25.61

[1] Knob nearest the end.
[2] Knob in second position from the end.
[3] Knob in third position from the end.

KNOB FREQUENCY

The number of knobs on the chromosomes may be the same in sister plants of a variety, but usually in varieties that have not been inbred the number varies somewhat from plant to plant, and this variation is frequently very pronounced when plants of varieties from different geographical regions are compared.

Not all knobs occur with the same frequency. Some knobs are usually present, whereas others are infrequently found.

The differences in frequencies led to an attempt to measure the frequency of occurrence of each knob in all the corn material studied. Since the data included a greater number of plants from varieties with few knobs on their chromosomes than from varieties with many knobs, it seemed best to group all plants according to the number of knobs present. This arrangement gave a grouping with classes ranging from 1 to 14. From this grouping the frequency of occurrence of each knob in each group was determined. The 14 determinations for each knob were then averaged to find the frequency of each knob in the whole population. Table 5 shows the frequencies determined by this method. This population does not include plants without knobs, but there were less than 1 percent of such plants.

TABLE 5.—*Percentage frequency of the 18 different chromosome knobs*

Chromosome		Percentage frequency in indicated number of plants having—															
No.	Arm	14 knobs (4) [1]	13 knobs (16)	12 knobs (23)	11 knobs (25)	10 knobs (23)	9 knobs (17)	8 knobs (11)	7 knobs (23)	6 knobs (25)	5 knobs (16)	4 knobs (24)	3 knobs (57)	2 knobs (75)	1 knob (26)	Mean
		Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
I	Short	100.0	100.0	100.0	92.0	95.7	100.0	100.0	87.0	72.0	75.0	83.4	87.7	96.0	69.2	89.3
V	Long	100.0	100.0	100.0	96.0	100.0	82.4	81.8	87.0	68.0	55.3	29.2	40.2	12.0	11.5	68.8
VII	do	100.0	100.0	100.0	92.0	91.3	76.5	90.9	69.6	76.0	43.7	16.7	14.5	9.3	3.8	63.2
IV	do	100.0	100.0	100.0	100.0	91.3	100.0	90.9	56.5	56.0	50.0	20.8	7.0	5.3	0	62.7
VIa [2]	do	100.0	81.2	79.1	80.0	95.7	52.9	27.3	43.5	40.0	50.0	75.0	59.7	48.0	15.3	60.5
II	do	100.0	100.0	83.3	100.0	78.3	82.4	72.7	47.8	56.0	37.5	37.5	22.8	5.3	0	58.8
VIIIb [3]	do	100.0	100.0	100.0	100.0	69.6	70.5	63.6	65.2	60.0	50.0	16.7	12.3	2.7	0	57.9
III	do	100.0	100.0	100.0	92.0	91.3	76.5	90.9	47.8	40.0	6.3	8.3	1.7	1.3	0	54.0
VIb [3]	do	75.0	81.2	83.3	60.0	73.9	47.0	36.4	39.1	24.0	25.0	58.3	21.0	10.7	0	45.3
II	Short	100.0	68.7	79.1	72.0	60.8	35.3	55.5	43.5	48.0	12.5	16.7	26.3	10.7	0	44.9
I	do	75.0	93.8	65.2	72.0	78.3	58.8	18.2	39.1	20.0	18.7	12.5	0	0	0	39.4
I	Long	50.0	68.7	69.6	48.0	13.0	41.2	18.2	21.7	12.0	6.3	0	0	0	0	24.9
VIIIa [3]	do	75.0	43.7	45.8	48.0	30.4	47.0	18.2	13.0	8.0	0	0	0	0	0	23.5
IX	do	100.0	100.0	50.0	4.0	8.7	11.8	9.1	4.3	0	12.5	8.3	1.7	0	0	22.2
III	Short	75.0	31.3	16.7	12.0	8.7	17.6	9.1	13.0	16.0	6.3	0	0	0	0	14.7
VIc [4]	Long	50.0	56.3	20.9	16.0	13.0	0	0	4.3	16.0	6.3	12.5	0	0	0	13.9
IV	Short	0	12.5	0	12.0	4.3	0	27.3	17.4	12.0	6.3	8.3	1.7	1.3	0	7.4
VII	do	0	0	4.2	4.0	0	0	0	0	0	0	0	0	0	0	.6

[1] Numbers in parentheses indicate number of plants.
[2] Knob nearest to the end.
[3] Knob in second position from the end.
[4] Knob in third position from the end.

KNOB-BEARING REGIONS

A few minutes' study of the 18 knobs shown on the chromosomes of figure 2 will be sufficient to convince the reader that they are not distributed uniformly over the whole length of the threads. There is an appreciable piece of each chromosome adjacent to the fiber attachment that is knobless. The fact that all knobs are found at some distance from the fiber attachment suggested that an attempt be made to determine the length of that portion on each chromosome arm in which knobs do not exist in this population of 365 plants. This portion of each chromosome will be considered as the knobless region.

The study of the relationship of knob position to knob frequency would have been much simpler had all chromosome arms been the same length. The long arms of chromosomes II, IV, VI, and VIII and the short arm of chromosome I, however, are approximately the same length and so, with some propriety, may be averaged. On these 5 arms there are 8 of the 18 known knobs, and these were used to make a preliminary test of the relationship between their frequencies and the positions they occupy on the chromosome arms.

These eight knobs are at positions 15.53μ, 24.17μ, 24.35μ, 25.96μ, 26.43μ, 27.18μ, 29.41μ, and 29.98μ from the fiber attachment (table 4). Their respective frequencies are 13.9, 45.3, 58.8, $\leftarrow\rightarrow$, 62.7, 57.9, 60.5, 39.4, and 23.5 percent (table 5). The increasing and decreasing array in knob frequency may be interpreted as a function of the distance from the fiber attachment. The frequency increases with distance until a point between 24.35μ and 25.96μ from the fiber attachment is reached; but from this point on, as the distance increases there is a decline in knob frequency. The point of highest knob frequency for arms approximately 36μ long is 25.96μ from the fiber

attachment. It is assumed that a knob located somewhere between 24.35μ and 25.96μ from the fiber attachment would occur with even higher frequency. Consequently, 25.2μ from the fiber attachment is taken as the knob position of maximum frequency. Knobs either side of this position will be found less frequently.

On the assumption that there is a real relationship between position and frequency of occurrence of knobs in the 5 chromosome arms of equal length, a similar relationship is to be expected for the 10 knobs on the remaining 15 arms.

Given the position of 25.2μ from the fiber attachment as the most favorable knob location on the five arms that are approximately 36μ long, the problem becomes one of determining where the corresponding position would be on longer or shorter arms.

The short arms of chromosomes X, VI, VII, and VIII (table 3) seem too short to reach a point at which knobs occur frequently. The next longer arm is the short arm of chromosome IX, which is 15.21μ long. This arm is terminated by a knob (table 4) that occurs with a high frequency (table 5), indicating that this point is close to the most favorable knob position on an arm of this length. Because a knob might occur with even a higher frequency than this terminal knob on the short arm of chromosome IX, it is assumed that a point will form a knob with maximum frequency at 15.2μ from the fiber attachment, a position very close to the end of this arm.

It has been shown above that arms longer by 20μ or more than the short arm of chromosome IX have their most favorable knob positions at approximately 25.2μ from the fiber attachment, a point 10μ farther from the fiber attachment than the terminal knob of chromosome IX. It follows that the most favorable knob position is farther removed from the fiber attachment for long than for short chromosome arms.

Many attempts were made to find a method for determining the expected location of the most favorable knob position with unit increase in arm length above 15.2μ. These attempts were governed by the two points already suggested, the one 15.2μ from the fiber attachment for a chromosome arm 15.21μ long, and the other 25.2μ from the fiber attachment for a chromosome arm approximately 36μ long. The increase of about 21μ in arm length above 15.2μ apparently has moved the position of maximum knob frequency only 10μ farther from the fiber attachment. The relationship between the increase in arm length above 15.2μ and the shift in position seems to be not a straight line but one that increases with each unit increase in length.

The following method was used to find the theoretical position of maximum knob frequency for each arm: Determine the number of microns each arm exceeds 15.2μ in length, square this excess, multiply it by 0.0225, and add the product to 15.2. This gives the position on each arm at which, if a knob-forming point exists, it will form a knob with greatest frequency.

The theoretical position on each of the 20 chromosome arms for maximum knob frequency has been determined by the above procedure and is given in table 6.

Figure 3 shows two positions, one on each arm of chromosome II. Figure 4 shows the curve (broken) that cuts each chromosome arm at the most favorable knob position. This curve does not touch the calculated position for the point on any of the 20 arms, though it does pass through one of the knobs, which of course is many times the

size of a point. In the following paragraphs it will be shown that the frequency of each knob is related to the distance of the knob from the point of maximum frequency as marked by this curve.

FIGURE 3.—Diagram of chromosome II, showing fiber attachment (circle), arms, knobless regions (solid black), favorable positions for knob-forming points (×), and knobs (solid black circles). ×1,500.

TABLE 6.—*Distance on each chromosome arm of most favorable knob position from fiber attachment*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
		μ	μ	μ			μ	μ	μ
X	Short	14.38	15.05	14.55	II	Short	19.61	20.35	19.81
VIII	do	14.66	15.45	14.85	V	Long	22.11	21.31	21.89
VI	do	14.75	15.58	14.96	VII	do	23.27	23.86	23.45
VII	do	14.86	15.54	15.03	I	Short	25.07	24.21	24.82
IX	do	15.00	15.83	15.20	VIII	Long	25.03	25.41	25.14
III	do	15.67	16.36	15.83	IV	do	24.95	25.87	25.23
IV	do	16.33	16.67	16.39	VI	do	26.55	23.86	25.70
X	Long	18.41	18.47	18.39	II	do	25.89	25.81	25.87
V	Short	18.41	18.95	18.53	III	do	30.41	31.73	30.75
IX	Long	18.68	19.60	18.91	I	do	37.59	36.44	37.28

Earlier it was stated that the short arms of chromosomes VI, VII, VIII, and X seemed too short to bear a knob of frequent occurrence. The longest of these four arms has a terminal knob that develops very rarely. This suggested that there is an area adjacent to the fiber attachment that is knobless. The longest of these four short arms extends to a point just slightly beyond this knobless area. This suggestion of a knobless area adjacent to the fiber attachment is supported by the fact that on no chromosome arm is the mean knob position nearer the fiber attachment than 12.44μ.

In determining whether the position of a knob on a chromosome arm affects its frequency, it seemed best to eliminate the knobless region adjacent to the fiber attachment from each arm.

To determine the length of the knobless region adjacent to the fiber attachment for each arm, the knob positions of two of the infrequently occurring knobs, one on a short arm and the other on a long arm, were used as a base. The knob terminating the short arm of chromosome VII has a frequency of 0.6 percent and is 2.59μ from the point calculated as that most favorable for knob formation. If moving the knob 2.59μ from the most favorable position decreases its frequency 99.4 percent, then its frequency should be zero at 2.60μ from the most favorable position, 12.43μ from the fiber attachment. In a similar manner, using the position and frequency of the knob nearest the fiber attachment on the long arm of chromosome VI, it is found that no knob would form at 13.89μ from the fiber attachment.

These two determinations suggested that the length of the knobless area adjacent to the fiber attachment increases with an increase in the length of the chromosome arm.

A method similar to that used in finding the most favorable knob position was used to find for each chromosome arm the length of the knobless region adjacent to the fiber attachment.

The data suggest that an arm shorter than 12.43μ would never bear a knob. The length of the knobless region for each arm was found by determining the number of microns each arm exceeds 12.43μ, squaring

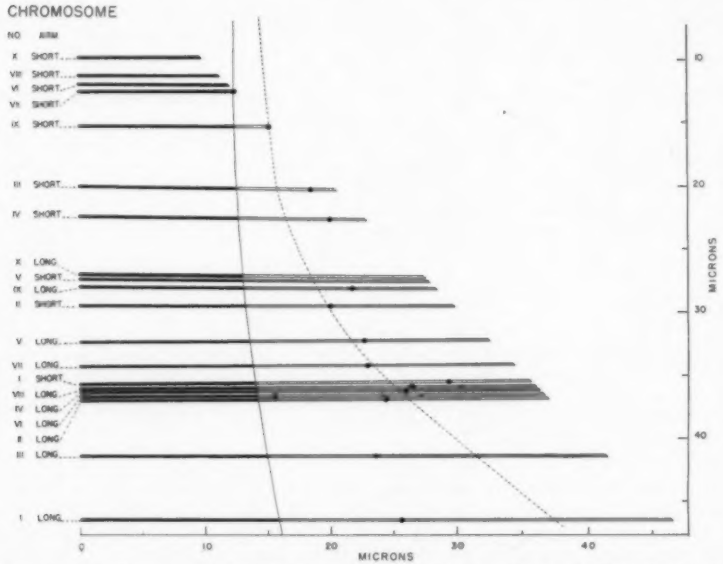

FIGURE 4.—Diagram of the 20 chromosome arms, showing the curve that cuts each arm at the distal end of the knobless region, the curve (broken) that cuts each arm at the most favorable position for a knob-forming point, and the knobs (solid black circles).

this excess, multiplying it by 0.00246, and adding the product to 12.43. By this method the length of the knobless region adjacent to the fiber attachment was found for each arm.

These lengths are given in table 7; figure 3 shows the knobless region adjacent to the fiber attachment for chromosome II; and figure 4 gives the curve that cuts each chromosome arm at the point that terminates the knobless region adjacent to the fiber attachment.

Fixing two points for each chromosome arm gives a piece of definite length on each. This piece lies between the distal end of the knobless region adjacent to the fiber attachment and the theoretical position on the thread of maximum knob frequency. Along this piece knobs will occur with a range of frequencies, depending on their position, from zero to a maximum, the latter for convenience being considered 100. The length of this portion of each chromosome arm is given in table 8.

TABLE 7.—*Length of the knobless part of each chromosome arm adjacent to fiber attachment*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
		μ	μ	μ			μ	μ	μ
X	Short	12.50	12.22	12.41	II	Short	13.20	12.99	13.14
VIII	do	12.51	12.22	12.43	V	Long	13.53	13.13	13.41
VI	do	12.51	12.22	12.43	VII	do	13.66	13.46	13.62
VII	do	12.51	12.22	12.43	I	Short	13.89	13.51	13.77
IX	do	12.52	12.25	12.45	VIII	Long	13.89	13.67	13.82
III	do	12.66	12.39	12.58	IV	do	13.89	13.72	13.84
IV	do	12.75	12.45	12.68	VI	do	14.06	13.46	13.89
X	Long	13.06	12.73	12.96	II	do	13.98	13.72	13.92
V	Short	13.06	12.80	12.98	III	do	14.53	14.47	14.51
IX	Long	13.08	12.90	13.03	I	do	15.39	15.05	15.28

This piece and an equal piece on the distal side of the maximum point make up the knob-bearing region of each chromosome arm. Figure 3 shows diagrammatically chromosome II and its fiber attachment. On each of its arms is shown the knobless region adjacent to the fiber attachment, the position theoretically most favorable for knob development, and the position of its knob.

TABLE 8.—*Distance on each chromosome arm from end of knobless region to most favorable knob position*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
		μ	μ	μ			μ	μ	μ
X	Short	1.88	2.83	2.14	II	Short	6.41	7.36	6.67
VIII	do	2.15	3.23	2.42	V	Long	8.58	8.18	8.48
VI	do	2.24	3.36	2.53	VII	do	9.61	10.40	9.83
VII	do	2.35	3.32	2.60	I	Short	11.18	10.70	11.05
IX	do	2.48	3.58	2.75	VIII	Long	11.14	11.74	11.32
III	do	3.01	3.97	3.25	IV	do	11.06	12.15	11.39
IV	do	3.58	4.22	3.71	VI	do	12.49	10.40	11.81
X	Long	5.35	5.74	5.43	II	do	11.91	12.09	11.95
V	Short	5.35	6.15	5.55	III	do	15.88	17.26	16.24
IX	Long	5.60	6.70	5.88	I	do	22.20	21.39	22.00

Referring to table 4, which shows the position of all knobs, it is possible to determine the distance of each knob from either the 0 or the 100 knob-frequency point. Table 9 gives the distance in microns of each knob from what is considered to be the most favorable knob position, namely, the 100 frequency point. Table 8 shows how many microns of chromosome length on each chromosome produce 100 points of change in knob frequency. From the figures of these two tables it is a simple problem to determine for each knob, from the position it occupies in respect to the 100 point, the reduction in frequency below 100. In table 10 these calculated reductions, converted into percentages, are given for all knobs. In table 11 is given the calculated frequency for each knob, derived by subtracting the reductions of table 10 from 100.

TABLE 9.—*Distance of each knob from most favorable knob position*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
		μ	μ	μ			μ	μ	μ
VII	Short	2.34	3.31	2.59	VIIIa [1]	Long	5.73	4.40	4.84
IX	do	.01	.02	.01	VIIIb [2]	do	1.63	.57	1.29
III	do	2.54	2.84	2.68	IV	do	.75	.73	.73
IV	do	3.36	3.11	3.62	VIa [1]	do	1.22	1.79	1.48
IX	Long	3.47	1.51	2.81	VIb [2]	do	1.66	1.51	1.53
II	Short	.95	1.51	1.18	VIc [3]	do	10.24	9.99	10.17
V	Long	.84	.57	.81	II	do	1.92	.80	1.52
VII	do	.44	.53	.48	III	do	6.52	7.96	7.10
I	Short	4.84	4.22	4.59	I	do	10.78	13.68	11.67

[1] Knob nearest the end.
[2] Knob in second position from the end.
[3] Knob in third position from the end.

TABLE 10.—*Distance of each knob from the most favorable knob position, expressed as percentage of distance from end of knobless part to most favorable knob position on the respective chromosomes*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No	Arm				No	Arm			
		Percent	*Percent*	*Percent*			*Percent*	*Percent*	*Percent*
VII	Short	99.57	99.70	99.61	VIIIa [1]	Long	51.44	37.48	42.76
IX	do	.40	.56	.36	VIIIb [2]	do	14.63	4.85	11.39
III	do	84.39	70.78	82.46	IV	do	6.78	6.01	6.41
IV	do	93.85	73.70	97.57	VIa [1]	do	9.77	17.21	12.53
IX	Long	61.96	22.54	48.81	VIb [2]	do	13.29	14.52	12.95
II	Short	14.82	20.52	17.69	VIc [3]	do	81.99	96.06	86.11
V	Long	9.79	6.97	9.55	II	do	16.12	6.62	12.72
VII	do	4.58	5.10	4.88	III	do	41.06	46.12	43.72
I	Short	43.29	39.44	41.54	I	do	48.56	63.95	53.05

[1] Knob nearest the end.
[2] Knob in second position from the end.
[3] Knob in third position from the end.

The calculated knob frequencies of table 11 agree with the observed frequencies of table 5. The two sets of frequencies are compared by means of the correlation of rank. The coefficient $\rho=0.959$, from a comparison of the observed frequencies and the calculated frequencies of the combined United States Indian and Mexican varieties, indicates the degree of the relationship between the frequency of a knob and its distance from a theoretical position most favorable for knob formation or less directly to its distance from the fiber attachment.

The foregoing discussion of the knob-bearing region of each corn chromosome considers only the data of the combined United States Indian and Mexican varieties. The Indian varieties and the Mexican varieties, however, were treated independently in a manner similar to that for the combined data, and these results also are given, in tables 6 to 11.

TABLE 11.—*Observed and calculated percentage frequency and order of frequency of each knob*

Knob location on chromosome—		Observed		Calculated					
				United States Indian varieties		Mexican varieties		Combined United States Indian and Mexican varieties	
No.	Arm	Frequency	Order of frequency	Frequency	Order of frequency	Frequency	Order of frequency	Frequency	Order of frequency
		Percent		*Percent*		*Percent*		*Percent*	
IX	Short	80.3	1	99.6	1	99.4	1	99.6	1
V	Long	68.8	2	90.2	5	93.0	6	90.5	4
VII	do	63.2	3	95.4	2	94.9	3	95.1	2
IV	do	62.7	4	93.2	3	94.0	4	93.6	3
VIa [1]	do	60.5	5	90.2	4	82.8	8	87.5	6
II	do	58.8	6	83.9	9	93.4	5	87.3	7
VIIIb [2]	do	57.9	7	85.4	7	95.1	2	88.6	5
III	do	54.0	8	58.9	10	53.9	13	56.3	12
VIb [2]	do	45.3	9	86.7	6	85.5	7	87.0	8
II	Short	44.9	10	85.2	8	79.5	9	82.3	9
I	do	39.4	11	56.7	11	60.6	12	58.5	10
I	Long	24.9	12	51.4	12	36.0	14	46.9	14
VIIIa [1]	do	23.5	13	48.6	13	62.5	11	57.2	11
IX	do	22.2	14	38.0	14	77.5	10	51.2	13
III	Short	14.7	15	15.6	16	29.2	15	17.5	15
VIc [3]	Long	13.9	16	18.0	15	3.9	17	13.9	16
IV	Short	7.4	17	6.1	17	26.3	16	2.4	17
VII	do	.6	18	.4	18	.3	18	.4	18

[1] Knob nearest the end.
[2] Knob in second position from the end.
[3] Knob in third position from the end.

DISCUSSION AND CONCLUSIONS

The nature and function of the bodies on corn chromosomes, known as knobs, are, as yet, an unsolved problem. The 18 known chromosome knobs have been shown diagrammatically in their mean positions in figures 1, 2, and 4. It is hardly necessary to point out again that these knobs are not distributed uniformly over the whole length of the chromosomes but are restricted in their distribution.

The absence of knobs on those portions of the chromosome threads adjacent to the fiber attachment made it possible to mark off on each chromosome arm a knobless region. The knobless regions are given definite limits in figures 3 and 4, but these may change as the studies of corn chromosomes progress. Two or three years ago several of the infrequently occurring knobs were unknown. At that time the knobless regions would have been longer than those given here.

The knob-bearing region of each chromosome arm increases as the knobless region decreases. The knob-bearing portion of each arm given in the preceding paragraphs fits only the present-day knowledge of chromosome knobs, and as soon as new knobs are found it will be extended, since it is considered that on the chromosomes of any plant 18 is the maximum number of knobs that will occur on the 20 knob-bearing regions herein described.

It is not unusual to find in some plants knobs on the chromosomes that are only slightly larger than the adjacent chromomeres, while in others they are prominent swellings on the thread. This variation in knob size led to the view that there exist 18 points on the chromosomes with the ability to organize knobs and that these points are constant in their position and are always present on the chromosomes of all

corn plants but are visible only when they become knobs. That other knob-forming points may exist outside the present defined knob-bearing areas will not be considered at this time, since this discussion is concerned primarily with the knob frequency at each of the 18 knob-forming points.

The data presented show that knobs on the knob-bearing regions do not occur with equal frequency and that the knob frequency increases as the distance of the knob-forming point from the end of the knobless region (or less directly from the fiber attachment) increases, until a certain position on the thread is reached beyond which the frequency of a knob at a knob-forming point decreases. This certain position on each chromosome arm is the position at which, if a knob-forming point exists, it will form a knob with greater frequency than a point located either nearer to or farther from the fiber attachment.

The knobless or the knob-bearing regions of the chromosomes may be found to change as studies advance, but the position where the knob-forming point forms a knob most frequently is thought to be at a fixed distance from the fiber attachment for each chromosome arm.

Knob formation depends primarily on the presence of a knob-forming point. At present 18 such points are known, all of which are thought to be present on the chromosomes of all corn plants. McClintock [4] describes a behavior of the nucleolus somewhat analogous to that described above where knob-forming points are shown to exist even when no knob is formed. McClintock found that nucleolar material at a certain stage is associated with all chromosomes but that later this material is drawn to a major organizing center or centers. In the case of knob-forming points, although no single point possesses a knob-forming power that overshadows all other points, there is a marked difference in the different points in their ability to form knobs.

It has been assumed that even the knob-forming point terminating the short arm of chromosome IX is slightly removed from the maximum knob-forming position on this arm, since its frequency is only 89.3 percent. None of the remaining 17 knob-forming points approaches this highest frequency; consequently there seems to be no case in which a knob-forming point coincides with a maximum position.

The differences in knob frequencies (table 5) show that knob-forming points are not alike in their ability to form knobs. It has been the purpose of this paper to show that the differences in knob frequency are related to a difference in position on the chromosome threads of each knob-forming point. The agreement between the observed knob frequencies and frequencies determined by position only has led to the conclusion that knob frequencies and knob positions are correlated.

The correlation between knob position and knob frequency is most readily apparent in chromosome arms of the same length. Most arms, however, are quite unequal in length. Consequently, a method had to be found for determining for chromosome arms of different lengths the position of two points, one having a zero and the other a maximum knob-forming ability. That these points are greater in linear distance from the fiber attachment when a chromosome arm

[4] McClintock, Barbara. The relation of a particular chromosomal element to the development of the nucleoli in Zea mays. Ztschr. f. Zellforsch. u. Mikros. Anat. 21: [294]–328, illus. 1934.

is long than when it is short is analogous to the behavior of two points, each an inch from the ends of a 3-inch rubber strap made to stretch unequally by having it one-fourth of an inch across at the base and tapering to one-sixteenth of an inch across at the top. If the base is held firm and the strap stretched from the top, each unit increase in length will change the relative positions of the two points either to the base or to each other. This change is similar to the change in position of the two points on the chromosome arm, one at the distal end of the knobless region and the other at the maximum knob-forming position, with each unit increase in length.

Since the data have shown that the ability of a knob-forming point to form a knob is related to its distance from the fiber attachment, it follows that each chromosome arm possesses a gradient. Darlington and La Cour [5] have shown something akin to this gradient in their statement that differential staining reaction "* * * seems to depend on distance from the centromere."

It is assumed that there is a gradient along each chromosome arm, which is equal in all at the fiber attachment and which increases toward the end, as measured by knob formation, until a point is reached where a knob-forming point will form a knob most frequently, after which it decreases. With a gradient of this sort it is possible to find a theoretical point on each of the 20 arms having equal power to form a knob, provided there exists at this position a knob-forming point.

It seems reasonable to conclude, from the data thus far accumulated, that no two knob-bearing points are at positions having equal power to form knobs and that the slight differences in position of the knob-forming points, when translated into a difference in gradient along the chromosome thread, account for the observed differences in knob frequency.

Since knobs are found also on the chromosomes of the close relatives of corn, it seemed likely that data on the frequency and position of knobs from these relatives would throw additional light on this problem of knob formation. A limited study of the Florida and Moyuta strains of annual teosinte (*Euchlaena mexicana* Schrad.) has provided only sufficient data to give an indication of the relationship between knob frequency and knob position in this related genus. These meager data show that the five shortest arms and the two longest arms are without knobs and that knobs at the end of arms having a certain length are present more frequently than are knobs at the end of arms, both longer and shorter. The maximum point for knob formation seems further removed from the fiber attachment than is the case on the arms of corn chromosomes.

In conclusion, if the ability of knob-forming points to collect knob material is controlled by a differential gradient along the chromosome thread, it seems reasonable to infer that other features of the chromosome are affected by this gradient.

The foregoing discussion goes no further than to propose that knob frequency is related to the position of a knob-forming point on the chromosome thread.

Position of a knob-forming point may not be the only factor controlling knob formation. An examination of the knobs on the chromo-

[5] Darlington, C. D., and La Cour, L. Differential reactivity of the chromosomes. Ann. Bot. [London] (n. s.) 2: 615–625, illus. 1938.

somes of corn from different regions clearly shows that the amount of knob material is not the same in all plants. Consequently, the 18 knob-forming points thought to be present on the chromosomes of all corn plants may not have, in different plants, the same amount of material for knob formation.

Assuming that the quantity of material available interacts with the position of knob-forming points to affect the frequency of knob formation, the following points are suggested for consideration in future studies:

(1) The amount of knob material varies from plant to plant.

(2) The number of knobs on the chromosomes of a plant is limited by the amount of material available. This material is usually insufficient to fill all knob-forming points.

(3) The position of a knob-forming point determines its ability to collect from the available knob material.

Consequently, the knob-forming point nearest a point of maximum knob-forming power will be filled with knob material most frequently and the point farthest from a point of maximum knob-forming power will be filled with knob material least frequently.

SUMMARY

A study of the midprophase of the first meiotic division of pollen mother cells from 74 varieties of corn of the United States and Mexico has shown that knobs are not distributed uniformly over the whole length of the chromosome but are at points on the thread at an appreciable distance from the fiber attachment.

The frequency of a knob has been shown to be a function of the distance of a knob-forming point from the fiber attachment.

The effect of the fiber attachment upon knob frequency is shown by the failure to find knobs in close proximity to the fiber attachment. The depressing effect of the fiber attachment on knob formation decreases as the distance from the attachment region increases, until a point is reached on each arm where knob formation is at a maximum. This change in knob-building power is interpreted to mean that each chromosome arm possesses a gradient.

A preliminary study of the Florida and Moyuta strains of annual teosinte reveals a relationship between the frequency of the knobs and the distance of the knob-forming points from the fiber attachment similar to that outlined for corn.

CATION EXCHANGE PROPERTIES OF CERTAIN FOREST SOILS IN THE ADIRONDACK SECTION [1]

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INTRODUCTION

The central Adirondack section in New York State offers an excellent opportunity for studying the properties of virgin forest soils. There are localities where the climax forest types have not been appreciably disturbed by man or fire. It is the purpose of this paper to present data on the cation-exchange capacity, exchangeable bases, percentage base saturation, percentage loss on ignition, and hydrogen-ion concentration for soils of similar geological origin but occurring beneath three distinct forest types, namely, red spruce, red spruce-sugar maple-beech, and sugar maple-beech-yellow birch. Furthermore, some general relationships are shown between organic-matter content, percentage base saturation, pH values, and the apparent pK values [2] of the soils.

DESCRIPTION OF FOREST TYPES

The study was conducted in the general vicinity of Newcomb, N. Y. The climate and geology of this locality have been adequately described elsewhere (*1, 7, 17*).[3]

The three forest types selected are briefly described as follows:

(1) Red spruce type. This is type 18 described in the report of the committee on forest types of the Society of American Foresters (*16*). It also corresponds to the *Oxalis-Cornus* type described by Heimburger (*7, pp. 32–33*). The type is composed chiefly of red spruce, with scattered specimens of yellow birch, balsam fir, red maple, beech, and hemlock.

(2) Red spruce-sugar maple beech type. This is type 17 in the Society of American Foresters' classification (*17*). It corresponds to the *Viburnum-Oxalis* type of Heimburger (*7, pp. 33–34*). It is composed chiefly of red spruce, sugar maple, yellow birch, beech, red maple, and scattered balsam fir and hemlock. This is the most common forest type in the central Adirondacks.

(3) Sugar maple-beech-yellow birch type. This is type 12 in the Society of American Foresters' classification (*16*) and more specifically corresponds to the *Arisaema* type of Heimburger (*7, pp. 36–37*). The general northern hardwoods type varies considerably in species composition. The type used here represents that on the richer sites in the Adirondack section. It is composed of sugar maple, beech, and yellow birch in varying proportions, with smaller admixtures of basswood, white ash, black cherry, and hophornbeam.

[1] Received for publication April 25, 1939.

[2] The apparent pK value is the logarithmic reciprocal of the dissociation constant of the soil acids. The method for its estimation is presented later.

[3] Italic numbers in parentheses refer to Literature Cited, p. 504.

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For convenience, in this paper, these three types will be referred to, in the order described, as the spruce type, spruce-hardwood type, and hardwood type.

The stands selected for study with respect to the influence of forest type upon exchangeable cations were essentially mature, virgin forests, with the exception that there had been a selective cutting of spruce and balsam on the spruce-hardwood type, thus decreasing the percentage of those species in the stands.

DESCRIPTION OF SOIL TYPES

In selecting the locations for obtaining soil samples for this study, the principal criterion was geological origin. The soils selected consisted of glacial till soils of similar texture in which Adirondack gneiss was the predominating rock. The profiles beneath the spruce and spruce-hardwood types were those of mature Podzol soils. The profiles beneath the hardwood types represented the Brown Podzolic soils. The humus layers [4] varied beneath the different forest types, there being a greasy mor under the spruce stand, a granular mor under the spruce-hardwood stands, and a fine mull beneath the hardwood stands. The essential morphological features of the profiles are presented in table 1.

TABLE 1.—*Description of glacial till soils used in this study*

Horizon	Spruce stand	Spruce-hardwood stand	Hardwood stand
A_0	Greasy mor humus layer about 8 inches thick with a black amorphous H layer [1] predominating.	Granular mor humus layer about 6 inches thick with black granular H layer predominating.	L and F layers about 1 inch thick H layer essentially absent. Fine mull humus layer.
A_1 (A_1 under hardwoods).	Gray, leached loamy sand, 3 inches thick.	Gray, leached loamy sand 3 inches thick.	Mixture of organic matter and mineral soil. Organic matter content over 30 percent. About 3 inches thick.
B_1	Very dark brown sandy loam. Relatively high in organic matter. Average thickness about 2 inches.	Dark coffee-brown sandy loam. High in organic matter, about 3 inches thick.	Dark chocolate brown loam, about 8 inches thick.
B_2	Rusty-brown sandy loam, about 5 inches thick.	Rusty-brown sandy loam, about 5 inches thick.	Yellowish-brown sandy loam about 10 inches thick.
B_3	Yellowish-brown sandy loam, about 7 inches thick.	Yellowish-brown sandy loam, about 8 inches thick.	Yellowish-gray sandy loam, about 9 inches thick.
C_1	Greenish-yellowish gray loamy sand. Very compact.	Greenish-yellowish gray loamy sand. Very compact.	Greenish-yellowish-gray loamy sand. Very compact.

[1] The terms, L, F, and H layer as used in this paper may be defined as follows: L layer is composed of freshly fallen leaves that have not appreciably decomposed; F layer is composed of litter in process of decomposition but which is still recognizable as to source; H layer is composed of litter that has decomposed to such an extent as to not be recognizable as to source. H is usually a black, finely divided, amorphous layer.

Two profiles were sampled beneath the spruce-hardwood and hardwood stands, but data were secured from only one profile beneath the spruce stand. The description is the average of all profiles beneath a given forest type. All sampling areas were within a radius of 10 miles of Newcomb. An inspection of table 1 reveals that the profiles beneath the spruce and spruce-hardwood stands

[4] Classification of Bornebusch and Heiberg (*3*).

were very similar, the principal difference being in the humus layer. The profiles beneath the hardwood stands were markedly different, being rich fine mulls, with an intense incorporation of organic matter in the A_1 horizon. The organic matter penetrated well into the B horizon and gave the soils a chocolate-brown color and a fine granular structure.

The soils beneath a single forest type varied very little from one locality to another. Two samples from a given forest type seemed adequate to characterize the soil beneath it. In fact, the correlation between vegetation and soils was so high that one could predict quite accurately the general type of profile from observation of the forest type and its characteristic ground vegetation.

The soil types have not been mapped in the Adirondack section, but the soils beneath the spruce, and the spruce-hardwood stands seemed to be closely related to the Beckett sandy loam type, while those beneath the hardwood stands corresponded to descriptions of Essex sandy loam type. Hence, two great soil groups were represented the Podzol and the Brown Podzolic.

In addition to the soils described above, some special studies were carried out on two other soils which were in the same general section, but which were coarser textured and were derived from glacial outwash. These soils corresponded quite well with those of the Colton series. They were mature humus Podzols and occurred beneath stands of the red spruce type.

In all cases a large soil pit was dug, so that the soil profile could be carefully examined. The samples secured were composites of soil taken from the sides of the pits.[5] The soils were air-dried and sifted through a 2-mm. sieve before analysis. All determinations were made on air-dry soil.

LABORATORY METHODS

The selection of methods for the determination of exchangeable cations in forest soils requires special attention because of the high content of organic matter in certain horizons. The cation, as well as the anion in the leaching salt, markedly affects the solubility of the organic matter. The ammonium ion is particularly active in this respect, and hence methods should be avoided which involve the treatment of soils with any ammonium salt previous to the determination of total exchange capacity. In order to demonstrate the effect of ammonimum acetate upon the reduction of the exchange capacity of forest soil samples, two organic layers and two C-horizon samples were treated in two different ways. One set was treated with neutral normal ammonium acetate solution, the exchangeable ammonium was replaced with calcium and the amount of exchangeable calcium determined to give exchange capacity. The other set was treated with 0.05 N hydrochloric acid and barium acetate solution in accordance with the procedure described later. The results are reported in table 2.

[5] The author is indebted to R. L. Donahue and H. T. Hopkins for obtaining certain of the samples.

TABLE 2.—*Comparison of ammonium acetate and hydrochloric acid treatments in determining exchange capacity of forest soils*

Organic matter (percent) in H layer and C horizon	Hydrochloric acid barium acetate treatment			Ammonium acetate—calcium acetate treatment		
	Exchange capacity per 100 gm.[1]	Exchangeable hydrogen per 100 gm.	Base saturation	Exchange capacity per 100 gm.	Exchangeable hydrogen per 100 gm.	Base saturation
	Milliequivalents	*Milliequivalents*	*Percent*	*Milliequivalents*	*Milliequivalents*	*Percent*
H layer, 88.6	148.2	128.7	13.1	130.6	127.8	2.1
H-layer, 81.8	158.0	135.7	14.1	129.0	134.5	
C-horizon, 0.93	2.6	2.0	23.1	2.6	2.0	23.1
C-horizon, 4.35	6.4	5.2	18.7	6.3	5.3	16.0

[1] Dry soil.

There was a decided reduction in exchange capacity of the H-layers when treated with ammonimum acetate. The C-horizons, being low in organic matter, were not appreciably affected. An effect similar to this would explain why Lunt (*8*) obtained higher values for exchangeable hydrogen than for exchange capacity in the organic layers of New England forest soils. It might also explain why Lutz (*9*) obtained percentage-base-saturation values of less than 10 for certain A_0 horizons with a pH value greater than 4.5.

In this study the hydrochloric acid-barium acetate method was used for determining the value for exchange capacity and exchangeable hydrogen. Two portions of a given sample were weighed out. One portion was used for the determination of the exchange capacity, while the other was used in the determination of exchangeable hydrogen. The exchange capacity was determined as follows: A sample of soil (1 to 10 gm. depending on the exchange capacity of the material) was weighed out and transferred to a 150-m. beaker. About 50 ml. of 0.05 N hydrochloric acid solution was added. The mixture was allowed to stand for several hours, with occasional stirring. Then the sample was filtered on asbestos, by using suction, and washed with successive 25-ml. portions of acid until 300 ml. had passed through the filter. The soil was then washed with water until the leachate was free from chlorides. The soil and asbestos were transferred to the beaker, and 50 ml. of neutral normal barium acetate was added. After several hours the mixture was again filtered and washed with 400 ml. of barium acetate, and the acetic acid in the leachate was titrated potentiometrically with 0.1 N barium hydroxide.

Exchangeable hydrogen was estimated by treating the soil directly with neutral normal barium acetate solution, and titrating the acetic acid as described above. The amounts of exchangeable bases were determined by obtaining the difference between exchange capacity and exchangeable hydrogen.

This procedure has the advantage of not dispersing the organic matter, and of permitting the titration of exchange capacity and exchangeable hydrogen to the same end point. The method is relatively rapid, as the final determinations are volumetric. Also, when either barium or calcium acetate are used, one cannot determine exchangeable calcium, but it can be determined in the 0.05 N filtrate hydrochloric acid filtrate secured in the determination of exchange capacity.

The pH value was determined potentiometrically in a thick soil-water suspension, a glass electrode being used.

Organic matter was estimated by loss of weight on ignition at a temperature of about 500° C. The values are doubtless slightly high, but since the soils were free from carbonates and contained less than 5 percent of clay, it seemed unnecessay to resort to more time-consuming methods. Hence organic matter is used in this paper as represented by the loss-on-ignition figures.

The pK values reported were calculated from the cation exchange data by the well-known formula:

$$pH = pK + \log \frac{\text{salt}}{\text{acid}}$$

Milliequivalents of bases represented salt and milliequivalents of hydrogen represented acid.

In order to check the accuracy of this procedure, 10 samples were half saturated with calcium hydroxide so as to cause the logarithm of the salt-acid ratio to become zero, and hence the pH value to become equal to the pK value. The results of these determinations were somewhat higher than those calculated directly (5.0 as compared with 4.8), but the correlation between them was high. The calculated values are reported in this paper.

The pK values give somewhat empirical figures for the strength of the soil acids, since the formula for their calculation is designed for use in simple systems involving a single monobasic acid. For this reason the constant is referred to in this paper as "apparent pK value." The results obtained, however, indicate that the constant is of considerable value in characterizing soils. The assumption is made that as the apparent pK value decreases, the dissociation of the soil acids increases. It is used to indicate the importance of the intensity as well as the capacity factor in soil-acidity studies.

EXPERIMENTAL RESULTS

In table 3 are presented the average data for the five profiles. In order to make certain relationships clear, the data are also presented graphically. The vertical scale for horizons assumes all layers to be of the same thickness. Also the graphs were drawn by connecting the points corresponding to the values determined for each horizon. This method of presentation was used to simplify the comparisons. If the actual horizon depths were plotted on the vertical scale and a block graph drawn for each horizon, it would be necessary to plot three graphs for each set of values and comparisons would be somewhat more difficult. An inspection of table 1 indicates that the depths of the B_1 horizons were essentially equal in all profiles; and since the values change gradually with depth, the straight lines probably represent the actual situation for the B and C horizons. No values are reported for the B_3 horizons because preliminary evidence indicated that the figures were always intermediate between those for the B_2 and C horizons and hence would not add any particular value to this report. To further simplify comparisons, the data for the A_1 horizon of the hardwood profile are plotted in the figures as though they applied to the A_2 horizon.

TABLE 3.—*Exchange capacity, exchangeable hydrogen, exchangeable bases, percentage base saturation, pH values apparent pK values, and percentage loss on ignition of soils beneath three forest types*

Forest type and horizon	Exchange capacity per 100 gm. air-dry soil	Exchangeable hydrogen per 100 gm. air-dry soil	Exchangeable bases per 100 gm. air-dry soil	Base saturation	pH	pK [1]	Loss on ignition
Spruce:	*Milliequivalents*	*Milliequivalents*	*Milliequivalents*	*Percent*			*Percent*
A_0 (H layer)	148.2	128.7	19.5	13.1	3.45	4.27	88.6
A_2	8.0	6.4	1.6	20.0	4.60	5.22	3.98
B_1	56.0	40.6	15.4	27.5	4.75	5.17	20.6
B_2	29.3	21.4	7.9	27.0	4.95	5.38	12.0
C_1	2.6	2.0	.6	23.1	5.05	5.53	.93
Spruce-hardwood:							
A_0 (H layer)	129.2	103.2	26.0	20.1	3.74	4.34	73.6
A_2	5.3	4.5	.8	15.1	4.03	4.75	2.25
B_1	48.9	38.8	10.1	20.7	4.35	4.93	13.8
B_2	31.8	22.7	9.1	28.6	4.86	5.25	14.0
C	3.3	2.3	1.0	30.3	5.27	5.63	2.01
Hardwood:							
A_0 (F layer)	122.5	33.8	88.7	72.4	5.56	5.15	79.7
A_1	55.8	29.5	26.3	47.1	5.05	5.10	33.3
B_1	30.8	19.7	11.1	36.0	5.14	5.39	17.0
B_2	27.2	17.9	9.3	33.8	5.24	5.52	12.5
C	4.7	3.1	1.6	34.0	5.32	5.60	2.8

[1] pK is the logarithmic reciprocal of the dissociation constant of the soil acids.

Figure 1 presents the data for total exchange capacity of the various horizons of the profiles beneath the three forest stands.

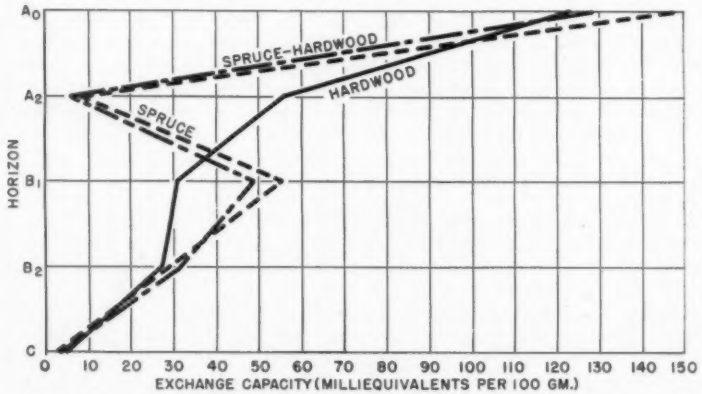

FIGURE 1.—Vertical distribution of total exchange capacity of the horizons of the profiles beneath the three forest types.

The values for the spruce and spruce-hardwood profiles were similar and were typical of humus-Podzol soils in the northeastern part of the United States. The hardwood profiles showed a different distribution, being somewhat higher in the leached horizon and somewhat lower in the B_2 horizon. These values were closely associated with the organic-matter content of the soils. This relationship is shown in figure 2 by plotting percentage loss on ignition against total exchange capacity. The line drawn through the dots was fitted in accordance

with the empirical curve formula for a straight line, $a+bx=y$, After solving for a and b, the formula became $-2.7+0.56x=y$. where x = total exchange capacity and y = percentage loss on ignition. The coefficient of correlation was 0.980. All samples used in this study were plotted individually. Figure 2 shows clearly that the organic matter is the seat of the exchange capacity of these Adirondack forest soils.

In order to indicate the magnitude of the organic exchange complex, the organic matter in the samples from one of the spruce-hardwood profiles was oxidized with hydrogen peroxide in accordance with the method of Olson and Bray (*14*). The exchange capacity of the

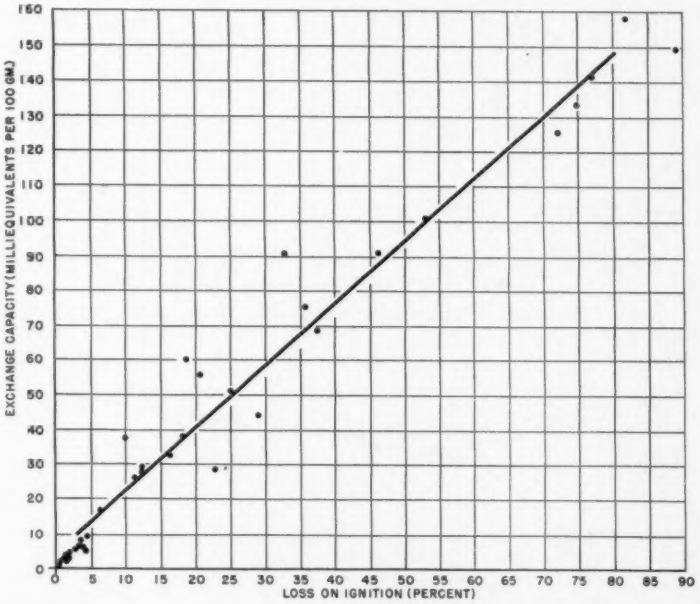

FIGURE 2.—Correlation between percentage loss on ignition and total exchange capacity for soils under hardwood.

H_2O_2-treated soil was determined. Figure 3 shows the curves for the natural and oxidized soils. This procedure does not accurately evaluate the organic-exchange capacity of the soils, as claimed by Olson and Bray (*14*), and Bartlett et al. (*2*). Meyers (*12*) has demonstrated that organic and inorganic exchange capacities are not additive. If the organic-matter content of a soil is very low, and the inorganic-exchange capacity is high, it is possible to increase the exchange capacity by removing the organic matter. Nevertheless, if one can assume that the exchange capacity of the inorganic fraction was not appreciably altered by the hydrogen peroxide treatment, the figure shows that the exchange capacity of the soil would be very low if the organic matter were not present.

The values for exchangeable bases are presented in figure 4.

Although the graphs show trends similar to those for total exchange capacity, there were certain important differences. The hardwood profile showed a very high concentration of bases in the A_0 and A_1 horizons. In the spruce and spruce-hardwood profiles there was a tendency for a slightly higher concentration of bases in the humus layer of the latter. Although there are only a few figures reported

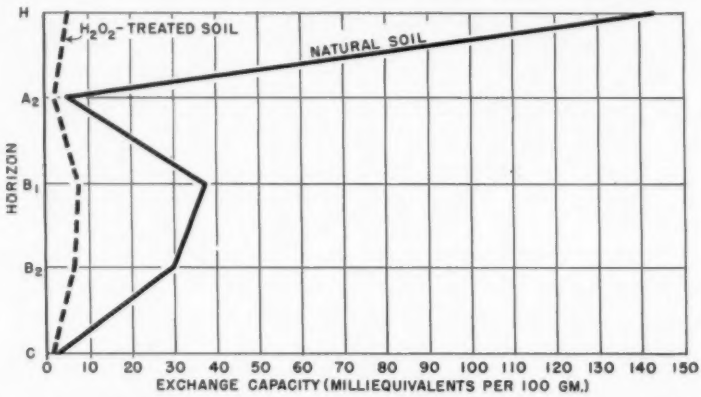

FIGURE 3.—The influence of removal of organic matter upon the exchange capacity of the various horizons of a Podzol profile beneath a spruce-hardwood stand.

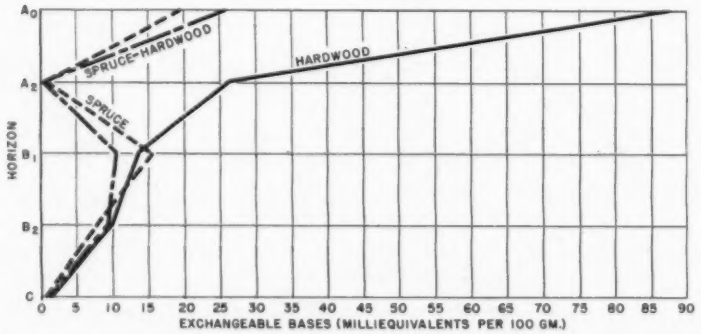

FIGURE 4.—Exchangeable bases in the various horizons of the profiles beneath the three forest types.

here, a large number of pH determinations made on other samples have shown this relationship to be consistent. These differences are most logically explained by the variation in the calcium content of the litter of the trees growing on the various sites. This matter will be treated further in the discussion of results. Because of the variability in exchange capacity among the various horizons, the values for exchangeable bases are better presented in the form of

percentage base saturation. These values, along with those for hydrogen-ion concentration, are plotted in figure 5.

The spruce and spruce-hardwood profiles exhibited similar trends. The hardwood profile showed a higher percentage base saturation as well as pH value in the surface soil although the differences in the B and C horizons were not large. The tendency was for percentage base saturation and pH value to increase with depth beneath the spruce and spruce-hardwood stands, while the opposite tendency

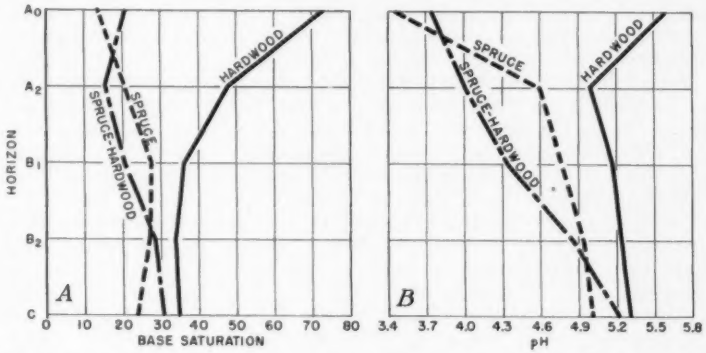

FIGURE 5.—Percentage base saturation (*A*) and pH values (*B*) of soils beneath the three forest types.

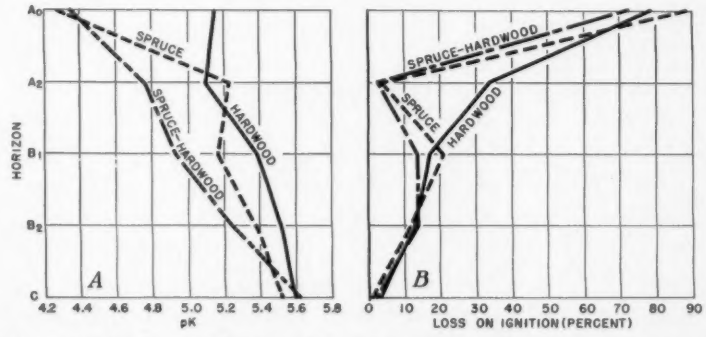

FIGURE 6.—Apparent pK values (*A*) and loss on ignition (*B*) for the various horizons of the profiles beneath the three forest types.

prevailed with the hardwood stand. The pH values increased with depth to a greater degree than did the percentage-base-saturation values. This increase was undoubtedly owing to the differences in strength of the soil acids and was associated with the organic-matter content of the soil. This point is supported by figure 6.

As the organic matter decreased, the apparent pK values tended to increase. This was not an exact relationship, and there were some exceptions to the rule. The A_0 horizon of the hardwood plots tended

to have a high apparent pK value, even though the organic-matter content was similar to the other A_0 horizons. This might be due, in part, to the fact that the degree of decomposition of the leaf litter was not so advanced. Furthermore, when the material becomes well decomposed in this type of soil, it is already mixed with the mineral soil. The apparent pK value for the A_2 horizon seemed rather low, in consideration of its organic-matter content. The inorganic-exchange capacity of the A_2 horizon was low, and what organic matter was present might have had a relatively great influence upon the salt-acid ratios at a given pH value. The steady increase in apparent pK value in passing downward from the B_1 horizon correlated well with the organic-matter content.

In certain humous Podzols on light-textured soils, the observation has been made that the pH value of fthe B_1 horizon was lower than that of the A_2. Such a profile has been described by Lyon and Buckman (*10*) and has caused some comment, because one naturally considers the A horizon to be more thoroughly leached than the B horizon and hence that it should be more acid.

Two such profiles from the Adirondack section were sampled. They were both strongly podzolized soils, originating from coarse sandy glacial outwash. Forest stands predominating in red spruce covered the sites. The organic-matter content of the B_1 horizon averaged 28.3 percent, that of the A_2 horizon only 1.69 percent; thus they were pronounced humus Podzols. The samples were analyzed for the same properties as for the profiles beneath the different stands. The results are reported in table 4.

TABLE 4.—*Average values for exchange capacity, exchangeable hydrogen, exchangeable bases, percentage base saturation, pH values, apparent pK values, and loss on ignition for two Podzol profiles derived from glacial outwash*

Horizon	Exchange capacity per 100 gm	Exchangeable hydrogen per 100 gm	Exchangeable bases per 100 gm	Base saturation	pH	Apparent pK	Loss on ignition
	Milliequivalents	*Milliequivalents*	*Milliequivalents*	*Percent*			*Percent*
A_0 (H layer)	140.6	114.2	26.4	18.8	3.65	4.38	70.0
A_2	3.35	2.67	.68	20.3	4.56	5.25	1.69
B_1	71.3	53.8	17.5	24.5	4.06	4.55	28.3
B_2	41.9	32.7	9.2	21.9	4.68	5.22	14.0
C	3.7	3.2	.5	13.5	4.61	5.41	2.4

In figure 7 are curves showing the trends for hydrogen-ion concentration, apparent pK values, and percentage base saturation.

Instead of the usual correlation between hydrogen-ion concentration and percentage base saturation, it was found that when the hydrogen-ion concentration increased, the percentage base saturation usually went down, and when they did increase together the increase in hydrogen-ion concentration was correspondingly greater than that for percentage base saturation. The apparent pK values showed the reason for this. The apparent pK value of the A_2 horizon showed a decided increase over that of the A_0 horizon. The pH value also increased about 1 pH unit. The percentage base saturation, however, increased only from 18.8 to 20.3 percent. The apparent pK

value dropped again in the B_1 horizon, and the percentage base saturation actually increased from 20.3 in the A_2 horizon to 24.5 in spite of the reduction in pH value from 4.56 to 4.06. It is the writer's belief that the difference in apparent pK value between the A_2 and B_1 horizons explains the occurrence of a lower hydrogen-ion concentration in the illuvial than in the eluvial horizon. The stronger acids in the B_1 horizon can bind more bases at a lower pH value than can the weaker acids in the A_2 horizon. All the profiles studied have consistently shown a greater degree of base saturation in the B_1 horizon than in the respective A_2 horizon regardless of the pH values. One would expect a lower hydrogen-ion concentration in the B_1 only in profiles which have a high accumulation of organic matter in the B_1 horizon and which have a very low inorganic-exchange capacity.

DISCUSSION OF RESULTS

The high content of exchangeable bases as well as the high percentage base saturation in the surface soil associated with the hardwood

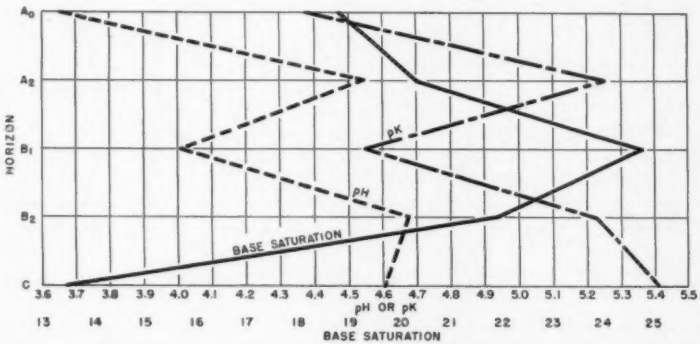

FIGURE 7.—Hydrogen-ion concentration, apparent pK values, and percentage base saturation for the sandy, glacial outwash, Podzol profiles.

stands was the most conspicuous influence of forest type upon the exchangeable cations in the soil. The fact that all the B and C horizons were so similar in this respect leads one to believe that the influence was truly associated with the forest type. It has been shown by several workers (*6*, *11*, *13*) that the calcium content of the tree leaves of different species may vary a great deal. That this calcium is important in influencing the pH value of the surface soil has also been demonstrated (*4*, *5*, *15*). In order to determine what relationship exists between the calcium content of the foliage of the trees and the exchangeable bases in the soil, leaf samples were secured from these trees in September 1938 and were analyzed for total calcium. Since the crown area of the trees is important in determining the amount of litter deposited and since basal area is closely correlated with crown area, the diameters of all trees in a circular area comprising 0.1 acre immediately surrounding the soil pit were taken and the data were expressed as percentage basal area by species. These

values were multiplied by the percentage calcium content of the foliage of each species, the summation of which gave an empirical figure that represented the relative amount of calcium deposited on a particular area. These data are presented in table 5.

TABLE 5.—*Proportion of the basal area of the 0.1 acre immediately surrounding the soil pit occupied by the different species along with the calcium content of the foliage and the relative amount of calcium added to the soil*

Type and species	Proportion total basal area occupied	Calcium content of foliage	Relative amount of calcium added to the soil[1]
Hardwood:	*Percent*	*Percent*	*Percent*
Sugar, maple	41.3	1.36	58.5
Basswood	23.2	2.82	65.2
Beech	20.1	.75	15.1
Yellow birch	8.9	1.34	11.7
Red spruce	0		
Hemlock	0		
Balsam fir	0		
Red maple	0		
American elm	1.9	2.00	3.8
White ash	4.6	1.91	8.8
Relative amount of calcium added to soil			163.1
Spruce-hardwood:			
Sugar maple	39.9	1.01	40.4
Basswood	0		
Beech	24.0	.60	14.4
Yellow birch	27.2	1.27	34.5
Red spruce	2.1	.87	1.8
Hemlock	6.3	.75	4.7
Balsam fir	.2	.96	.2
Red maple	.3	.95	.3
American elm	0		
White ash	0		
Relative amount of calcium added to soil			90.3
Spruce:			
Sugar maple	0		
Basswood	0		
Beech	5.0	.63	3.1
Yellow birch	25.4	1.21	27.9
Red spruce	61.3	.85	52.1
Hemlock	2.1	.71	1.6
Balsam fir	3.2	.96	3.1
Red maple	3.0	.84	2.5
American elm	0		
White ash	0		
Relative amount of calcium added to soil			96.3

[1] Obtained by multiplying the percentage basal area by the figures for calcium content of foliage.

The relative value of 163.1 for the hardwood stand is very much larger than the values of 96.3 and 90.3 for the spruce-hardwood and spruce stands. The low value for the spruce-hardwood stand as compared with the hardwood stand was caused by the absence on the former area of such high-calcium species as basswood, American elm, and white ash. In addition, the trees of the same species tended to absorb considerably more calcium on the hardwood sites. This was especially true with respect to sugar maple. Although exchangeable calcium was not determined on many samples, preliminary results, as well as those obtained by other workers, indicated that exchangeable calcium represented from 75 to 90 percent of the total bases.

These calculations assume, of course, that the trees deposited amounts of litter in proportion to their basal areas and hence can only be considered as rough estimates. But the trend is doubtless well indicated by the figures. The data in table 5 show the fallacy of the common assumption that the foliage of all hardwoods has a high calcium content as compared with that of coniferous trees.

The results obtained in this study tie in with previous studies made in the same section. Hopkins [6] has recently shown that the concentration of tree roots is highly correlated with the moisture equivalent and percentage loss on ignition of these same soils. Donahue [7] has demonstrated the greater productivity of the hardwood sites as compared with the spruce-hardwood and spruce areas. Heimburger (7) has shown that the soils under this hardwood type nitrify much more than do soils under the other two types. It is the writer's opinion that these differences in productivity are caused largely by the influence of the type of litter deposited annually.

The question naturally arises, however, as to why different forest types occur on soils presumably identical at the end of the last glacial epoch. This is a moot question, to which there are several possible answers:

(1) The distribution of the trees may have been accidental originally, but once established they continued to reproduce themselves and made the site more or less favorable, depending on the species present.

(2) There may be differences in the soil characteristics which have not been discovered, as yet.

(3) The water conditions as determined by topography may have been influential in determining the distribution of tree species.

This last point seems to be rather likely, although it has not been proved. Observation of some 20 soil pits in the Adirondacks during the season of 1938, which happened to be an exceptionally wet one, revealed that the pits in the hardwood sites always had a certain amount of moving ground water passing through them, and that during extremely rainy spells a free water table was present on top of the compact C horizon. This situation did not prevail on the spruce-hardwood or spruce sites. At no time was there a perched water table in the soils in these sites. There were no apparent differences in permeability of the parent material. The influence evidently arose from the topographical features of the areas, the hardwood sites always being situated near the base of a long slope, with an opportunity for extra water to move down the hillside. If extra water is available on these sites during wet periods, in all probability the areas would not suffer so seriously during dry periods. This point needs further study. But if such is the case, the difference in species composition might be due to the fact that during periods of drought some of the more exacting species such as basswood, white ash, and even sugar maple were eliminated from the sites where sufficient water did not exist. It should be mentioned here that the spruce-type profile was not a very characteristic one, because the majority of the spruce stands in the Adirondacks occur on either lighter-textured soils and/or soils that are poorly drained and have a high, stagnant water table. Therefore the foregoing discussion applies more particularly to the hardwood and spruce-hardwood types.

[6] HOPKINS, H. T. ROOT DISTRIBUTION OF TREES IN THE ADIRONDACK REGION. M. S. thesis, Cornell Univ. 1939.

[7] DONAHUE, R. L. FOREST GROWTH AND SOIL MORPHOLOGY IN THE CENTRAL ADIRONDACK REGION. Ph. D. thesis, Cornell Univ. 1939. (In preparation.)

SUMMARY

On soils of similar geological origin, the sugar maple-beech-yellow birch forest type produced a soil type which, in the surface layers, was less podzolized, had a higher pH value, and a larger percentage base saturation than soil profiles beneath stands of the red spruce—sugar maple—beech type, or the red spruce type. The differences between the latter two types were small.

Evidence is submitted to indicate that the cause of these differences was associated with the calcium content of the foliage of the trees.

A high correlation existed between total exchange capacity and percentage loss on ignition. Percentage base saturation and pH value were highly correlated when soils of similar organic matter content were compared.

The apparent pK values of the various horizons indicated that the soil organic matter had a lower apparent pK value than that of the mineral soil. The values were somewhat higher for the hardwood profiles, indicating that weaker acids were involved.

The lower apparent pK value of organic matter and hence the higher percentage base saturation at a given hydrogen-ion concentration, explained why it was possible to have a lower pH value in the B_1 horizon than in the A_2 horizon of a humus podzol profile.

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RETENTION OF SOME PHOSPHORUS COMPOUNDS BY SOILS AS SHOWN BY SUBSEQUENT PLANT GROWTH[1]

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INTRODUCTION

That soils strongly retain the orthophosphates is well recognized. These phosphates applied in dry condition move only very short distances with percolating waters. Applied in solution as in laboratory studies or under field conditions in the irrigation water, the orthophosphate ions are removed from solution and retained by the solid phase of the soil. Little is known, however, of the reactions of many other phosphorus compounds with the soil.

In connection with other studies in progress, it became desirable to know approximately how much certain organic phosphorus compounds were retained by soils and to ascertain the effect of each on plant growth. Orthophosphates as well as other inorganic phosphorus compounds were included for comparison.

METHODS

The usual method of attack in this type of investigation is to study by chemical analysis each compound in turn, its reaction with the soil, and then to determine its physiological reaction on plant growth as a separate test, later correlating the findings from the two types of tests. A more direct method was used, however, which placed its main reliance upon the physiological reaction of the plant. This method, described by Conrad and Adams (*3*),[2] is illustrated in figure 1. The plants by their enhanced growth showed the position of the phosphorus retained from a percolating solution by a soil deficient in phosphorus, if the compound in question was beneficial. If the compound was toxic, reduced growth was one criterion of retention used. The cations, calcium and sodium, it is assumed in these studies contributed little or nothing to the beneficial or toxic properties of the compounds tested.

Two lots of soils deficient in phosphorus were used in these experiments: Yolo subsoil (a loamy fine sand), C–11, collected at a depth of 4 to 8 feet from Yolo fine sandy loam on the south bank of Putah Creek, University Farm, near Davis, Calif. (*4*); and Aiken loam, C–22, a surface sample from near Paradise, Calif. (*12*).

Each 4-inch pot, previously coated with asphaltum paint, was provided with a square of waxed paper to cover the hole. Dry soil (400 gm. per pot) was then added to each. The three pots of each column were stacked with the bottom one nesting in the drainage can (No. 2½ cannery tin also painted on the inside to prevent rusting) and with the consecutive pots held apart by a cannery tin top punched

[1] Received for publication Apr. 17, 1939. Investigations of the Division of Agronomy, California Agricultural Experiment Station, Davis, Calif. The analyses reported herein were made by R. E. Malde, Division of Agronomy.

[2] Italic numbers in parentheses refer to Literature Cited, p. 517.

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with a large hole (fig. 1). The reservoirs shown were not used in securing the data for tables 1 and 2; but they were employed for later studies. In the early work the solutions in question were poured into the top pots in successive amounts as percolation progressed.

In the comparisons made below it is assumed that practically an equal amount of liquid was retained by each pot in a given column. To test the validity of this assumption, pots were weighed both before and after percolation, and the leachings measured before being discarded or stored for analysis. Table 1 summarizes these data and gives estimates of their statistical significance. The amount of water held by a soil may, as noted elsewhere (*11, p. 16*), continue to increase

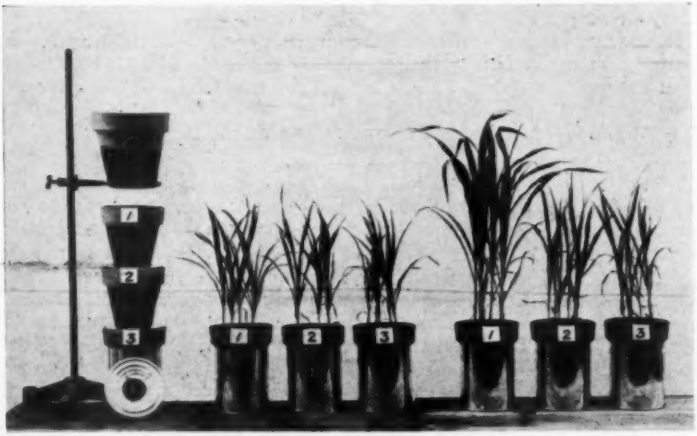

FIGURE 1.—Retention of the phosphorus from percolating solutions. At *A* the solution being tested slowly dripped from the reservoir above, down upon the column of 4-inch pots (1, 2, and 3), each containing 400 gm. of dry soil (Aiken loam) deficient in phosphorus. After percolation the pots in the column were taken down and subsequently cropped to milo. Equal amounts of nitrogen were added to each pot in the test during the growth period. The three pots at *B* were percolated with distilled water, those at *C* with a solution of Na_2HPO_4.

for several hours as wetting of the aggregates with excess water continues. This principle explains why the water held by the middle pots was a few percent higher than that held by the bottom pots. Undoubtedly the top pots held a little more than the middle ones originally, but because of evaporation from their unprotected surfaces, the amount is unknown. With the Aiken soil in the heated greenhouse, greater evaporation would be expected from the top pots than with the Yolo soil, columns of which were percolated in the unheated head house. The water held by the top pots in these experiments was evidently less than 10 percent more than that held by the bottom ones.

TABLE 1.—*Amount of percolating solution retained by the soil in the pots of the columns*

Item		Yolo subsoil, C–11	Aiken loam C–22
Columns	number	18	39
Solution added per column	milliliter	465	675
Solution retained:			
Pot No. 1, top	grams	145	198
Pot No. 2, middle	do	[1] 142	[2] 209
Pot No. 3, bottom	do	137	199
Leachings, bottom pots	do	20	32
Accounted for:			
Milliliters		444	638
Percent		95.5	94.5

[1] Statistically different from the value below it. P=0.01 (*5, p. 112*).
[2] Statistically different from the value above or below it. P much less than 0.01 (*5, p. 112*).

The phosphorus compounds used and the grades were as follows:

Calcium orthophosphate primary, $Ca(H_2PO_4)_2.H_2O$; c. p.
Sodium orthophosphate primary, $NaH_2PO_4.H_2O$; c. p.
Sodium orthophosphate secondary, $Na_2HPO_4.12H_2O$; c. p.
Sodium pyrophosphate, $Na_4P_2O_7.10H_2O$; c. p.
Metaphosphoric acid, HPO_3—glacial sticks
Sodium hypophosphite, $NaH_2PO_2.H_2O$; c. p.
Calcium glycerophosphate; N. F.
Sodium phosphite, $Na_2HPO_3.5H_2O$
Sodium hypophosphite; N. F. V.
Calcium hypophosphite; N. F.
Sodium glycerophosphate
Sodium β-glycerophosphate
Triethyl phosphate, $(C_2H_5)_3PO_4$

Sodium nucleate (stock solution) was made by dissolving 8.01 gm. of yeast nucleic acid in 62.5 ml. of M/2 NaOH and diluting to a liter.

EXPERIMENTS

The results of the first tests are reported in table 2. All phosphorus-containing solutions reported therein contained 2 milligram-atoms of phosphorus for each column of three pots. Since milo (*Sorghum vulgare Pers.*) seed had been planted in the dry soil, germination started with percolation. During the growth period 5 milligram-atoms of nitrogen as urea were added to each pot of the Yolo subsoil, and a total of 9 milligram-atoms as $Ca(NO_3)_2$ and urea to each pot of the Aiken loam. As soon as practicable the plants were thinned to six per pot. For the Yolo soil the leachings from each column were analyzed for total phosphorus. Only traces were found in any, except from the $Ca(H_2PO_2)_2$ columns. Here a solution containing 4.3 milligram-atoms of phosphorus per liter was added at the top, and the leachings at the bottom contained 3.14 milligram-atoms per liter. After 48 days for the Yolo soil and 38 for the Aiken, the green crops were harvested, weighed, dried at about 80° C. for 48 hours, and reweighed. The averages of triplicate cultures are reported in table 2.

TABLE 2.—*Retention of phosphorus from various percolating solutions by phosphorus-deficient soils in columns of 4-inch pots as shown by the subsequent average yield of triplicate cultures of milo*

YOLO SUBSOIL (A LOAMY FINE SAND), C–11

Pot No.	Yield per pot (grams) from percolating solution indicated							
	Control, H_2O (distilled)		$Ca(H_2PO_2)_2$, N. F.		Calcium glycerophosphate		$Ca(H_2PO_4)_2$, c. p.	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1, Top	8.94	1.46	4.44	[1] 0.63	14.53	[2] 2.48	[3] 19.11	3.28
2, Middle	8.61	1.50	4.98	[2] .63	15.26	[2] 2.60	9.88	1.66
3, Bottom	8.63	1.57	5.79	[2] .81	13.04	[2] 2.34	8.81	1.51

AIKEN LOAM, C–22

	Control, H_2O (distilled)		$Ca(H_2PO_2)_2$, N. F.		Calcium glycerophosphate		Phytin	
1, Top	4.08	0.84	3.43	0.69	14.30	[2] 2.70	3.78	0.73
2, Middle	3.87	.81	4.01	.79	3.05	.64	3.60	.72
3, Bottom	4.27	.89	3.36	.68	4.23	.92	3.90	.78
	NaH_2PO_4, c. p.		$NaHPO_4$, c. p.		$Na_4P_2O_7$, c. p.		Na_2HPO_3	
1, Top	15.71	[2] 2.87	18.07	[2] 3.61	10.31	[4] 1.87	3.54	0.63
2, Middle	4.50	.91	4.08	.77	3.77	.74	4.30	.72
3, Bottom	4.57	.96	3.91	.76	3.90	.82	4.63	.85
	NaH_2PO_2, N. F.		Sodium glycerophosphate		Sodium β glycerophosphate		Sodium nucleate	
1, Top	3.09	[2] 0.57	10.06	[4] 1.79	10.12	[4] 1.77	8.50	[4] 1.47
2, Middle	4.28	.80	4.06	.76	3.87	.71	4.46	.87
3, Bottom	4.09	.76	4.45	.84	3.85	.77	3.46	.76

[1] Statistically different from the control; P lies between 0.05 and 0.01 (*5, p. 114*).
[2] Statistically different from the control; P=0.01 or less (*5, p. 114*).
[3] Statistically different from the value below it; P=0.01 or less (*5, p. 118*).
[4] Statistically different from the value below it; P lies between 0.05 and 0.01 (*5, p. 118*).

The Yolo soil retained the hypophosphite ion to only a slight degree, as shown by the toxic condition that extended rather uniformly throughout the soil column, each pot yielding significantly less than the control. The analyses of the leachings as reported in the text above are in agreement with this view. The phosphorus of the glycerophosphates penetrated significantly through the columns of the Yolo soil and into the bottom pots, but not sufficiently to evidence any increase of phosphorus in the leachings. The phosphorus of calcium orthophosphate was retained in the top pots.

From the Aiken loam columns as reported in table 2, the leachings were measured but not analyzed. Phytin, $Ca(H_2PO_2)_2$, and Na_2HPO_3 gave no significant differences from the controls. On the other hand, NaH_2PO_2 gave a significantly lower yield in the top pots than in the pots next below. The phosphorus of NaH_2PO_4, Na_2HPO_4, $Na_4P_2O_7$, and the sodium glycerophosphates as well as that of sodium nucleate was retained in the top pots by the Aiken soil, since significantly higher yields were secured there.

Some of the results given in table 2 were not decisive. If a chemical containing phosphorus gives an increase on a soil deficient in that element, it may be inferred that the phosphorus therein is responsible for the increase even though the chemical is not highly purified. When a phosphorus compound not of a highly refined grade gives a decrease on such a soil, we cannot so readily infer that the phosphorus compound is responsible, since the toxicity may be caused by some impurity of a highly toxic nature. For the work reported in table 2, hypophosphite of c. p. grade was not available; but some was secured for the work reported in later tables.

Other chemicals giving somewhat inconclusive results were used in higher concentrations than before, and the amounts of the percolating solutions increased to give ample leachings for analytical work. For growth reported in table 3, 10.4 milligram-atoms of nitrogen were added to each pot as mixed calcium, magnesium, and sodium nitrates.

TABLE 3.—*Retention of the phosphorus from various percolating solutions by phosphorus-deficient soils as shown by the subsequent yield of milo and the amount of phosphorus absorbed by the plants*

[Each value in the table is the average of triplicate cultures and analyses]

Pot No.	Water							
	Yolo subsoil, C–11				Alken loam, C–22			
	Yield weights		Phosphorus, dry	Phosphorus in crop	Yield weights		Phosphorus, dry	Phosphorus in crop
	Wet	Dry			Wet	Dry		
	Grams	*Grams*	*Percent*	*Milligrams*	*Grams*	*Grams*	*Percent*	*Milligrams*
1, Top	4.21	0.89	0.06	0.54	3.38	0.68	0.06	0.41
2, Middle	3.95	.80	.057	.46	3.63	.73	.065	.48
3, Bottom	3.86	.80	.06	.48	3.55	.75	.06	.45
Leachings (milligram-atoms of P per liter)		.006				.006		
	Phytin, 4[1] milligram-atoms							
1, Top	[2]8.94	[2]1.44	0.17	[2]2.45	4.96	0.94	0.08	0.75
2, Middle	4.00	.84	.09	.76	3.22	.65	.07	.45
3, Bottom	4.05	.84	.07	.58	3.31	.66	.065	.43
Leachings (milligram-atoms of P per liter)		.03				.01		
	HPO_3, 8[1]				NaH_2PO_2, c. p., 4[1]			
1, Top	4.47	0.75	0.21	1.58	3.66	0.62	0.11	0.68
2, Middle	9.68	1.41	.197	[1]1.78	3.72	.66	.10	[3].66
3, Bottom	4.18	.88	.08	.70	3.76	.73	.055	.40
Leachings (milligram-atoms of P per liter)		[3].03				[3].006		
	$Na_4P_2O_7$, c. p., 4[1]				$Ca(H_2PO_4)_2$ c. p., 4[1]			
1, Top	[1]7.72	[1]1.28	0.33	[1]4.23	[1]24.73	[1]3.23	0.30	[1]9.69
2, Middle	3.80	.79	.07	.55	3.79	.78	.07	.55
3, Bottom	4.11	.83	.07	.58	4.05	.81	.07	.57
Leachings (milligram-atoms of P per liter)		[3].02				[3].006		

[1] Milligram-atoms of phosphorus.
[2] Statistically greater than the value below it. P=0.01 or less (*5, p. 118*).
[3] Statistically greater than the value below it. P lies between 0.05 and 0.01 (*5, p. 118*).

Just before harvest, it was evident that the growth of the test plants alone would not conclusively give the location of the phosphorus in all the columns. In the most inconclusive cases the plants were weighed, dried, weighed, and then analyzed for total phosphorus. The results are reported in table 3 as "Phosphorus in crop." The concentration of phosphorus in the leachings is also given. In all cases reported in table 3 except the controls, the concentration of phosphorus in the leachings was not greater than 0.5 percent of that in the percolating solution.

The Yolo soil retained the phosphorus of phytin and of $Na_4P_2O_7$ in the top pots. The phosphorus of metaphosphoric acid, HPO_3, penetrated in statistically significant quantities to the second pot, with a suggestion of penetration to the third, as shown by the analyses of the crops grown on them. The growth from the top pot was practically equal to that with distilled water and was less than half that of the second pot. The yield alone is not a true criterion of the location of the phosphorus. There is no rational means by which all the phosphorus could pass through the top pot and become concentrated in the middle one. The most obvious explanation is that either hydrogen ions or metaphosphate ions or the resultant products of the reactions of each or both in the soil were retained in sufficient quantity in the top pot to just balance the toxic and beneficial effects.

TABLE 4.—*Retention of the phosphorus from various percolating solutions by phosphorus-deficient soils as shown by the subsequent green growth of milo and the phosphorus content of the leachings collected from the bottom of the columns*

YOLO SUBSOIL, C-11

Pot No.	Yield per pot (grams) from percolating solution indicated [1]						
	H_2O [2]	Na_2HPO_3 [3] (12.9)	NaH_2PO_3 [3] (6.45)	$(C_2H_5)_3PO_4$ [3] (12.9)	H_2O [4]	$(C_2H_5)_3PO_4$ [4]	
						0.97	4.83
1, Top	4.21	[5] 1.45	[5] 0.58	[5] 0.00	4.22	2.54	[6] [7] 0.10
2, Middle	3.95	[6] .98	[6] .61	[6] .00	4.25	2.46	[6] [8] .73
3, Bottom	3.86	[5] 1.25	[6] .74	[6] .32	3.97	4.00	2.19
Leachings per liter	[1] .006	[1] 2.9	[1] 6.3	[1] .022			

AIKEN LOAM, C-22

Pot No.	H_2O [2]	Na_2HPO_3 [3] (8.9)	HPO_3 [3] (8.9)	$(C_2H_5)_3PO_4$ [3] (8.9)	H_2O [4]	$(C_2H_5)_3PO_4$ [4]	
						0.67	3.33
1, Top	3.38	[5] [8] 1.72	[7] 11.41	[5] 0.00	2.29	2.06	[5] 0.88
2, Middle	3.63	3.65	3.72	[6] .00	2.37	1.92	[7] 1.10
3, Bottom	3.55	3.63	2.41	[6] .00	2.17	1.92	[8] 1.16
Leachings per liter	[1] .006	[1] .006		[1] .29			

[1] As milligram-atoms of phosphorus per liter.

[2] Mixed calcium, magnesium, and sodium nitrates added; 10.4 milligram-atoms of nitrogen per pot.

[3] Mixed nitrates added; 10.0 milligram-atoms of nitrogen per pot. 3 pots each for Yolo subsoil averaged 2.65 gm. and for Aiken loam, 2.41 gm. These received no phosphorus and were not subject to percolation, but otherwise were treated like those reported above.

[4] Mixed nitrates added; 7.4 milligram-atoms of nitrogen per pot. In this series of tests 1 column of each soil was percolated with H_2O; 2 with the lower and 3 with the higher concentrations of $(C_2H_5)_3PO_4$ (triethyl phosphate). All other values in this table are the average of triplicate cultures and analyses.

[5] Statistically different from the control; P lies between 0.05 and 0.01 (*5, p. 114*).

[6] Statistically different from the control; P=0.01 or less (*5, p. 114*).

[7] Statistically different from the value below it; P=0.01 or less (*5, p. 112*).

[8] Statistically different from the value below it; P lies between 0.05 and 0.01 (*5, p. 112*).

Aiken loam did most obviously retain the phosphorus of phytin, but in a form only slightly, if at all, accessible to the test plants. The yields of the top pots, however, just failed to be significantly higher than those of the middle ones (P=about 0.06). The c. p. grade of NaH_2PO_2 did not significantly show its toxicity for milo on Aiken loam. The analyses of the plants, however, gave significant evidence that the phosphorus of this compound had penetrated into the second pot of the column.

In table 4 the data for Na_2HPO_3 and NaH_2PO_2 with the Yolo soil and for the Na_2HPO_3 with the Aiken soil were obtained at the same time as those for table 3. Subsequent trials, as reported in table 4, were with various concentrations of triethyl phosphate on both soils and with HPO_3 on Aiken loam. Some of the responses in growth to

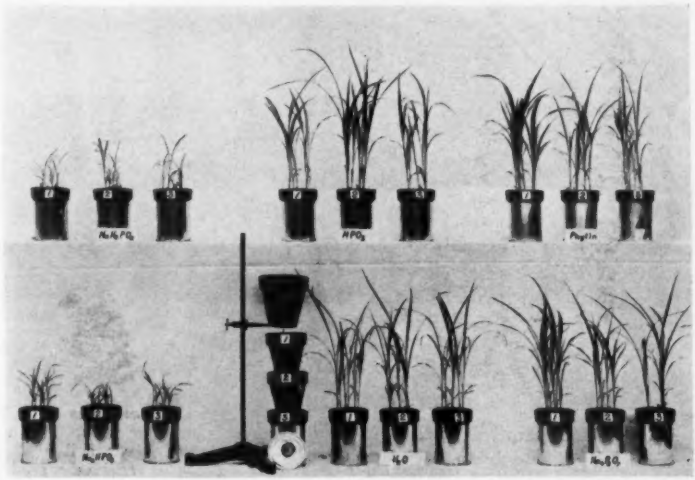

FIGURE 2.—Retention of phosphorus from various solutions by Yolo subsoil, C–11. Enhanced growth in some cases and reduced growth in others were important criteria of retention (cf. tables 3 and 4).

various phosphorus-containing solutions reported in tables 3 and 4 are shown in figure 2 for Yolo subsoil and in figure 3 for Aiken loam.

The evidence shows that the phosphorus of Na_2HPO_3 was retained to some extent by the Yolo soil, since the concentration of phosphorus in the leachings was but 25 percent of that of the original solution. The phosphorus of NaH_2PO_2 was retained hardly at all. The toxicity of Na_2HPO_3 and NaH_2PO_2 extended significantly down through the soil columns.

With Aiken loam the evidence indicates that most of the Na_2HPO_3 was retained in the top pot. The phosphorus of HPO_3 did not significantly penetrate beyond the top pot, though there was a suggestion (P=0.2) that some had reached the middle pot. Triethyl phosphate was retained to a certain extent by both soils, but somewhat more strongly by the Yolo soil. It was, furthermore, toxic to plant growth

—more so on the Aiken then on the Yolo soil, especially with the highest concentrations used.

DISCUSSION

Table 5 gives an estimate of the final percentage distribution of the phosphorus of the original percolating solution, assuming that the solvent was distributed in four equal parts among the three pots and the leachings. Increases and decreases in yield, the analyses of the

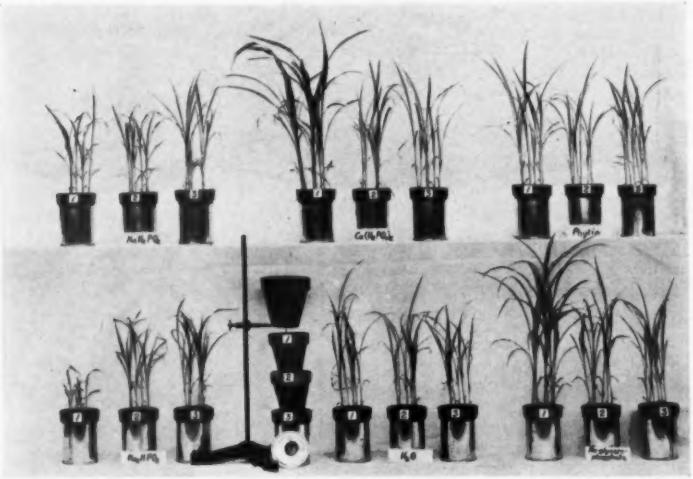

FIGURE 3.—Retention of phosphorus from various percolating solutions by Aiken loam, C–22. Retention is judged in part by the enhanced or reduced growth of milo in the top pots (cf. tables 3 and 4). The lot of Aiken soil used for sodium glycerophosphate and for the corresponding controls received a slightly different nutritional treatment. The top, middle, and bottom pots of the controls averaged for green weights 4.40, 4.77, and 4.81 gm. respectively, while those for the sodium glycerophosphate averaged 18.34, 4.88, and 4.54 gm. respectively. The yields from the top pots of the sodium glycerophosphate columns differed from the others very significantly. None of the other differences, however, were significant.

leachings, and the principle that no pot could retain a higher percentage of the original phosphorus than a pot above it in the same column were used as bases of estimation. Undoubtedly, reestimating of the data would result in slightly different figures in a few cases, but the relationships would not be materially changed. The possibility of more accurately estimating the location of the phosphorus compounds, by increasing the number of pots in the column was not explored in this study.

TABLE 5.—*Summary of retention data as estimated final percentage distribution of the phosphorus of the original solutions*

Chemical source	Percentage retention of phosphorus by—							
	Yolo subsoil, C–11				Aiken loam, C–22			
	Pot No. 1	Pot No. 2	Pot No. 3	In leachings	Pot No. 1	Pot No. 2	Pot No. 3	In leachings
NaH_2PO_2	28	26	24	22	70	30	0	0
Na_2HPO_3	40	30	23	7	90	10	0	0
HPO_3	75	25	0	0	90	10	0	0
$Na_4P_2O_7$	100	0	0	0	100	0	0	0
Na_2HPO_4					100	0	0	0
NaH_2PO_4					100	0	0	0
$Ca(H_2PO_4)_2$	100	0	0	0	100	0	0	0
Glycerophosphates	38	36	26	0	100	0	0	0
Phytin	100		0	0	100		0	0
Triethyl phosphate	45	35	20	0.1	43	35	21	1
Sodium nucleate					90	10	0	0

In seeking to discover the mechanisms by which the different phosphorus-containing units are retained one should consider the following factors:

(1) *Precipitation in the soil.*—If the anions of the percolating solution form a very difficultly soluble salt with any cations that might be liberated from the soil colloids and made soluble by exchange with the cations of the original solution, precipitation would be expected. Although many salts are theoretically possible, from a practical standpoint few besides the calcium and magnesium salts require attention. Since no hypophosphites are insoluble, the fact that the phosphorus was nearly equally divided in the Yolo subsoil would be expected. Since the hypophosphites were retained rather strongly by the Aiken soil, some other mechanism must be sought. Calcium and magnesium phosphites are only slightly soluble. From a qualitative standpoint, then, some of the retention of phosphites might be by precipitation. Since the Yolo soils are neutral and the Aiken soils are distinctly acid (about pH 5.8), it is difficult to explain the greater retention of phosphites by the Aiken soil on the basis of precipitation alone. Calcium metaphosphate is difficultly soluble, whereas the magnesium salt is soluble. Retention might be expected from the formation of the calcium salt by cationic exchange. The same might be said for the pyrophosphates and orthophosphates.

Since none of the glycerophosphates likely to be formed in the soil are insoluble, the strong retention of the phosphorus of glycerophosphates by the Aiken soil must be explained on other grounds.

Phytin, the calcium (sometimes some magnesium is present) salt of phytic acid, is soluble. Nucleic acid itself is listed as insoluble in water (7), and magnesium nucleate is also given as insoluble. Retention of the phosphorus of sodium nucleate, as shown by plant growth, might then be due to the formation of magnesium nucleate as the sodium exchanges with magnesium, a replaceable cation.

Triethyl phosphate, an ester of ethyl alcohol and phosphoric acid, miscible with water in all proportions, would not have a chance, as far as we know, to form an insoluble salt if it remained unchanged.

(2) *Adsorption or anionic exchange.*—Undoubtedly, other major factors in the retention of the orthophosphate ions by soils might be the phenomena of anionic exchange between the percolating solution and the solid phase of the soil and adsorption of the whole molecule by the solids in the soil as suggested by work reviewed by Russell (*9*) and more clearly indicated by later evidence (*1, 2, 8*). The data herein show some retention of the triethyl phosphate, an ester without ionic properties. Unless marked hydrolysis of this compound has taken place during percolation, this phenomenon suggests adsorption of this ester as whole molecules by the solid phase of the soil. To what extent the same types of phenomena were effective in causing retention of pyrophosphates, metaphosphates, phosphites, and hypophosphites is as yet unknown. If the hypophosphites remain as such, adsorption (or anionic exchange) seems to be the only process by which these phosphorus-bearing units could be retained. If such widely different compounds as hypophosphites (with Aiken loam) and orthophosphates with soils generally are adsorbed, one may suppose that compounds with intermediate properties could also be so retained.

(3) *Transformations to retainable chemical forms by hydrolysis, oxidation, or other chemical change.*—The possibility of the change from one form to another during the actual process of percolation cannot be neglected. If a mechanism for such a transformation (during the time when the solution itself is moving through the soil) exists in one soil and not in another, differences in behavior might necessarily result. More evidence is necessary before this factor can be given major consideration. The hypothesis that either the hypophosphites or the phosphites are transformed to orthophosphates and retained as orthophosphates, seems untenable because of the toxicity of the reduced forms of phosphorus as contrasted with the enhanced growth resulting from the phosphates, unless, of course, at the same time other toxic materials are formed thereby.

Some readers may question whether the differences in retention of the various phosphorus compounds found in these two soils may not be attributable to a difference in soil class since one is a loamy fine sand the other a loam, and the loam, which has a higher amount of colloid, is for the most part more retentive. Undoubtedly, this is a factor of importance. Two pieces of evidence point, however, to other factors as well. The much lighter soil, the Yolo, retained triethyl phosphate more readily than did the Aiken soil, and the crop on the Yolo was less injured. Again, 75 percent of the glycerophosphate was leached by Spencer and Stewart (*10*) from a 7-inch depth of Las Vegas loam with a 24-inch depth of water. This is the same type of behavior observed with the Yolo soil; but the Aiken soil, by retaining the glycerophosphate in the top pot, seems to exemplify another type of behavior.[3] This difference in behavior may later be associated with a difference in the type of soil colloids or with other factors as yet not evaluated.

[3] While this manuscript was being prepared a paper by Hilbert *et al* (*6*) came to the writer's attention. Using the methods of analytical chemistry on the leachings, they found differences similar to those here reported in the behavior of glycerophosphates on various soils. The writer's Yolo subsoil, like their Norfolk sandy loam and Las Vegas loam, retained the phosphorus of the glycerophosphates only weakly, whereas the Aiken loam, like their Cecil clay loam, retained it very strongly.

The data presented herein answer in a qualitative or semiquantitative manner certain questions concerning the interaction of these phosphorus compounds with the soils studied. The evidence, however, serves to raise many other questions, especially regarding the various mechanisms that may be responsible for the results secured. Without a doubt, the reactions of each chemical with the soil might very well be made the subject of a special detailed study.

SUMMARY

Each phosphorus compound in solution was allowed to percolate through a stack or column of three pots containing dry soil deficient in phosphorus. The volume of solution was sufficient to wet all the soil, but without great excess. If the compound was beneficial, the enhanced growth of the subsequent crop of milo was considered as evidence of retention; if toxic the reduced growth was one criterion of retention. Analyses of the crop and of the leachings were other criteria sometimes used.

Of the inorganic compounds tested, Yolo subsoil showed little retention of hypophosphites, but somewhat more for phosphites. The phosphorus of metaphosphoric acid reached the second pot, but that of the pyro- and orthophosphates was held in the top pots. The Aiken soil for the most part held each inorganic compound more strongly than did the Yolo.

Of the organic compounds tested, the Yolo soil retained the phosphorus of phytin in the top pot, but held back the glycerophosphates only slightly. The Aiken soil retained the phosphorus of nearly all these compounds as well as that of sodium nucleate, in the respective top pots. Triethyl phosphate, held back somewhat by both soils, was more strongly retained by the Yolo and was more toxic on the Aiken.

The phosphites and hypophosphites were toxic to milo. The meta-, pyro-, and orthophosphates (if not too concentrated) and the glycerophosphates were beneficial to both soils, with phytin beneficial on the Yolo soil and sodium nucleate on the Aiken soil.

The possible mechanisms by which the various compounds were retained are discussed, including chemical precipitation in the soil, either adsorption or anionic exchange or both, and chemical transformations (during the actual process of percolation) to some other forms retained by the soil.

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PRODUCTION OF GROWTH SUBSTANCE ON PEPTONE BROTH BY CROWN GALL BACTERIA AND RELATED NONGALL-FORMING ORGANISMS [1]

By S. B. Locke, *formerly research assistant in plant pathology*, A. J. Riker, *professor of plant pathology*, and B. M. Duggar, *professor of botany and plant pathology, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

The physiology of crown gall development has been the subject of numerous studies in the general field of pathological cell growth. The recent work with phytohormones has stimulated investigation about their relation to crown gall development. The ability of the crown gall organism, *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. to produce growth substance when cultured on certain media (*1*, *3*, *8*)[3] has been demonstrated, and more than the normal amount of growth substance was found in gall tissue (*9*). Thus, the possibility that the growth substance, if produced abundantly in the tissues, may be the cause of the pathogenicity, as suggested, for example, by Nemec (*12*), Brown and Gardner (*3*), and Link, Wilcox, and Link (*8*), and as claimed by Berthelot and Amoureux (*1*), appeared worth further study.

The production of beta-indole-acetic acid in bacterial cultures is no recent discovery. Long before it was identified with a plant-growth substance (*7*) it was isolated from bacterial cultures by a number of workers (*5*, *15*). Following the development of the *Avena* technique for quantitative estimation of this product (*16*) several workers undertook the study of growth-substance production by bacteria (*2*, *14*). These investigations indicate that the ability to produce growth substance in culture is widespread among such organisms. Thus it seemed that a study of the production of beta-indole-acetic acid by virulent crown gall bacteria in comparison with that by attenuated crown gall and nonpathogenic bacteria might shed light on the relation of this substance to pathogenicity. This paper, of which a preliminary report was given earlier (*10*), presents the results of experiments on the relative abilities of virulent crown gall bacteria, attenuated crown gall, and *Bacillus radiobacter* Beij. and Van Deld. to produce growth substance in peptone broth.

METHODS

The organisms employed were the progeny of single-cell isolations. The virulent crown gall (A6) and attenuated crown gall (A6–6) are sister cultures obtained from a single-cell virulent culture (*4*). The response of tomato (*Lycopersicum esculentum* Mill.) to inoculation with the attenuated culture is very slight. While the responses of several other plants, e. g., *Bryophyllum pinnatum* (L.) Kurz, *Kalanchoe*

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[2] The writers are indebted to Eugene Herring for preparing the illustration.
[3] Italic numbers in parentheses refer to Literature Cited, p. 524.

daigremontiana Hamet and Perrier, and *Sedum spectabile* Bor., are greater than that of tomato to the attenuated form, they are always much less than the response of the same hosts to the virulent culture (*11*). *Bacillus radiobacter* (R3–1) was included because it resembles *Phytomonas tumefaciens* physiologically, but produces no overgrowths whatsoever when inoculated into the plants mentioned above.

The medium employed in the cultures of the organisms was made up as follows: Parke, Davis' Bacteriologic Peptone, 10 gm.; Armour's Beef Extract, 3 gm.; mannitol, 5 gm.; and distilled water, 1,000 cc. In one case (trial 3), 40 mg. of l-tryptophane per liter was added. The hydrogen-ion concentration was adjusted to pH 7.0 with N/20 NaOH and the medium was distributed in 250-cc. portions into 1-liter Erlenmeyer flasks which were then plugged and sterilized for 1 hour at 15 pounds pressure.

The flasks were seeded with a 3-mm. loopful from a 48-hour tube culture on the medium described. The cultures were carried in duplicate in each trial and two unseeded flasks served as controls. The cultures were incubated at laboratory temperature in all but the final trial, in which incubation was at 23°–24° C.

Determinations of growth substance were made in red light at 23°–25° C. and over 90-percent relative humidity in accord with Went's standard technique (*17*). Beginning shortly after seeding and extending through a period of 2 or three weeks, the samples were drawn at intervals aseptically from each flask. Without concentration, each sample was mixed with an equal volume of melted 3-percent agar and the mixture was poured into a mold 1 by 8 by 11 mm. from which 12 equal blocks were cut. These were placed unilaterally on 12 decapitated *Avena* coleoptiles which were prepared as follows: The oats, variety State's Pride, Pedigree 7–7, were placed in water at 9 o'clock each morning. After 1 to 2 hours the water was poured off and the oats were hulled and placed, embryo up, upon an aluminum wire screen which covered a small dish filled with water so that the screen touched the surface of the water at all points. The seeds were allowed to germinate in red light at 23°–25° C. until the second morning, when the coleoptiles were about 5 mm. long. They were then placed in glass holders, 12 to a rack, and, with their roots in water, were allowed to develop 1 day longer. At this time those having coleoptiles between 25 and 30 mm. in length were selected and decapitated about 2 mm. below the tip. Three hours later they were decapitated a second time and the first leaf was pulled loose from the base and left projecting about 5 mm. from the top of the decapitated coleoptile. The agar blocks containing the growth substance were placed on one side of the decapitated coleoptile, being supported in this position by the projecting first leaf. Good contact between block and coleoptile stump was obtained by touching the bottom of the block with a small drop of water. Ninety minutes after applying the blocks a shadow print of each rack of coleoptiles was made. When growth substance was present in the blocks the coleoptiles curved away from the side on which the block was applied because of the greater growth of that side of the coleoptile. This curvature was measured on the shadow prints by means of a protractor and the average curvature of 12 coleoptiles was taken as a measure of the growth substance present in a given preparation. In the final trial a standard solution of known activity was employed

daily to determine the sensitivity of the coleoptiles on that particular day (*13*, *17*). In this case it was possible to express the results in gamma per liter of beta-indole-acetic acid.

EXPERIMENTAL RESULTS

The 4 trials reported in table 1 were made during a period extending from January 1 to September 17, 1938, and involved the use of 5,160 coleoptiles. In every trial, growth substance was produced in considerable quantity in all three cultures, while curvatures obtained with the unseeded medium were relatively small. Differences among curvatures obtained with samples from the different cultures were too small to be considered significant. Since the rate of production and the time required to reach a maximum concentration were different in the several trials, the results of each series are discussed separately. The summary of data obtained in these trials are given in table 1. In addition to the 4 series mentioned above, 3 other trials were made involving the use of 2,112 coleoptiles. While these gave results paralleling those reported, they are omitted for the sake of brevity and because of slight modifications in procedure.

TABLE 1.—*Summary of 4 trials in which the production of growth substance on peptone broth by Bacillus radiobacter, attenuated crown gall bacteria, and virulent crown gall bacteria was determined*

Trial No.	Culture	Negative curvature obtained after indicated number of days[1]															
		0	1	2	3	4	5	6	7	9	10	11	14	17	19	21	24
		°	°	°	°	°	°	°	°	°	°	°	°	°	°	°	°
1	Unseeded medium				9				1		1		1	1			4
	Bacillus radiobacter				12				6		6		14	18			27
	Attenuated crown gall				9				9		7		15	18			27
	Pathogenic crown gall				8				9		11		18	25			27
2	Unseeded medium	2			4					5			3		1		
	Bacillus radiobacter	4			6			25		26			27		28		
	Attenuated crown gall	5			7			26		26			26		29		
	Pathogenic crown gall	4			8			23		24			32		25		
3	Unseeded medium		0	2	2	1	1	2	0	1	1	2	4				
	Bacillus radiobacter		4	6	5	8	18	16	16	9	9	12	8				
	Attenuated crown gall		6	7	7	9	16	16	10	11	8	12	13				
	Pathogenic crown gall		4	2	5	10	14	16	11	10	12	12	14				
4	Unseeded medium		1		3	3	3		3	2		3	1	1		4	
	Bacillus radiobacter		2		4	5	11		8	6		8	9	9		12	
	Attenuated crown gall		2		2	5	8		9	8		5	8	8		10	
	Pathogenic crown gall		1		3	2	6		6	5		6	8	12		11	

[1] Each figure represents the average of curvatures produced in 12 *Avena* coleoptiles.

Trial 1 was begun January 31 and was completed February 24, 1938. Following a slight decrease in growth-substance curvatures from the third to the seventh day there was a gradual increase throughout the duration of the experiment, a maximum of about −27° being reached on the twenty-fourth day after seeding, when the experiment was terminated. Curvatures produced by unseeded medium dropped to near −1° on the seventh day and remained there throughout the rest of the experiment except for the final determination, when a curvature of approximately −4° was obtained.

Trial 2 was begun March 7 and concluded March 26. Growth-substance curvatures in all three cultures increased slowly during the first 3 days to about −7°, then rapidly until the sixth day to about −25°, and then very slowly until a maximum of about −30° was

reached on the nineteenth day when the experiment was terminated. The curvatures produced by the unseeded medium were close to −5° for the first 9 days, then gradually decreased to nearly 0° on the nineteenth day.

Trial 3 was begun August 1 and concluded August 15. To the basic medium, 40 mg. of l-tryptophane per liter was added. After a lag period of 3 days the curvatures produced by all three cultures increased rapidly to a maximum of about −18° and −16° on the fifth and sixth days respectively. This was followed by a decrease to about −10° in the next 3 days, and then by an increase to about −12° by the eleventh day. On the fourteenth day the crown gall cultures gave about −13° while the *radiobacter* culture gave a curvature of about −8°. This is not considered a significant difference, especially since it may be attributed to the fact that the determination was made just after the activity of the *Bacillus radiobacter* culture had dropped, but shortly before a similar decrease in activity occurred in the crown gall cultures. The unseeded medium gave curvatures of less than −4.5° throughout the experiment.

Trial 4 was begun August 27 and terminated September 17. The curvatures produced by the cultures showed a lag period of from 3 to 4 days, which was followed by a period of rapid increase reaching a maximum of approximately −6° to −11° on the fifth day. From the seventh to the ninth there was a decrease in the curvatures to between −5° and −6°. This was followed by a more gradual increase in curvatures to about −11° on the twenty-first day when the experiment was discontinued. The curvatures produced by the unseeded medium at no time exceeded −4.5°. A standard solution was tested on each lot of coleoptiles employed during this trial so that it is possible to express the activities of the samples in terms of concentrations of beta-indole-acetic acid giving the same curvatures (fig. 1).

While of minor importance, the bimodal character of the curves obtained in trials 3 and 4 is of interest, and can be explained in several ways. For example, while the cultures are young they probably draw mainly upon the sugar present as a source of carbon, which has a sparing action on the peptone. However, as the cultures age they probably use the nitrogenous compounds as sources both of carbon and of nitrogen. These changes in nutrition could bring about a change in growth-substance concentration (1) by altering the reaction of the medium and thus the stability of the growth substance, or (2) by altering the kind and quantity of compounds present from which the growth substance could be formed.

The possibility was considered that the differences in pathogenicity of the organisms employed may be attributed to qualitative rather than quantitative differences in the growth substances produced. So, the stabilities of these growth substances toward acid and base were tested. The growth substances from all three cultures proved to be unstable in acid and stable in basic solution, indicating that they belong to the indole acids, probably beta-indole-acetic acid (*6*).

DISCUSSION AND CONCLUSIONS

No significant differences were found among the three organisms employed with respect to their ability to produce growth substance when grown on peptone broth, notwithstanding the fact that they

differ widely in their ability to induce overgrowths when inoculated into plants susceptible to crown gall. The possibility still remains that the formation of growth substance by the crown-gall organism from

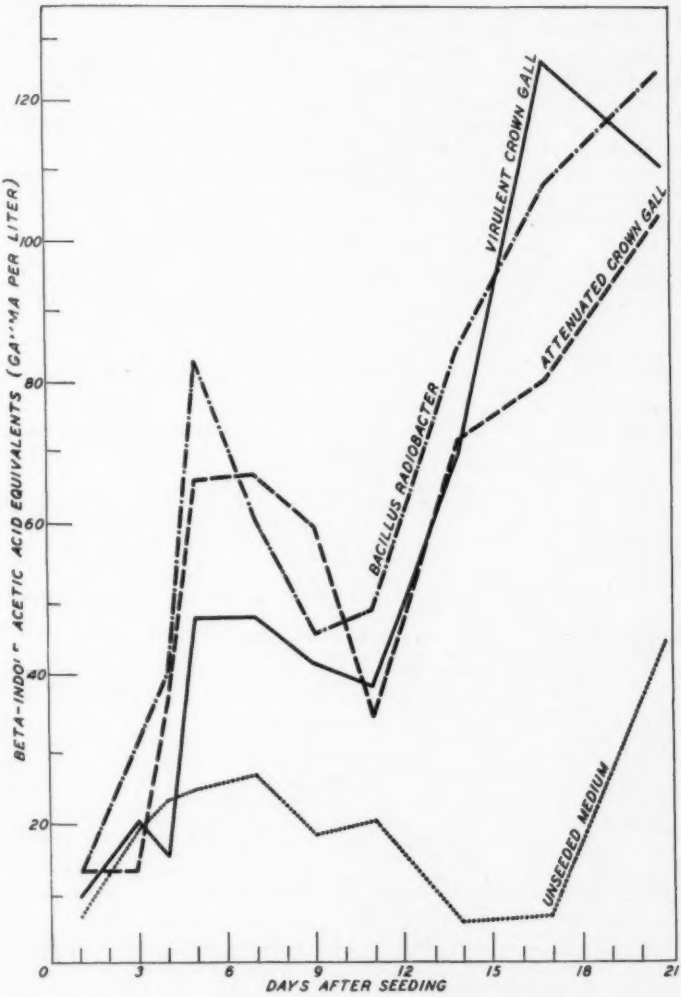

FIGURE 1.—Heteroauxin concentration in unseeded peptone broth and in virulent crown gall, attenuated crown gall, and *Bacillus radiobacter* cultures.

the materials available to it within the host tissues may constitute a part of the mechanism leading to excessive growth. However, the possession of this ability does not yet explain the differences in pathogenicity among the organisms employed in these experiments. As pointed out earlier (*9*, *11*), the difference in pathogenicity between the virulent and the attenuated crown-gall cultures seems not to be ex-

plained by a differential bacteriostatic effect of the host. Consequently, evidence seems lacking that the growth substance produced in culture has a direct major relation to the pathogenicity of crown gall-bacteria.

SUMMARY

Single-cell bacterial cultures of three organisms (virulent crown gall, attenuated crown gall, and *Bacillus radiobacter*) differing widely in ability to induce overgrowths in plants, but similar in physiology, were found to be similar in their capacity to produce growth substance in peptone broth. Thus far evidence seems lacking for the view that beta-indole-acetic acid or any other known growth substance produced in such cultures has a direct major relation to the pathogenicity of crown-gall bacteria.

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SPORULATION AND VIABILITY OF OÖCYSTS OF EIMERIA ARLOINGI FROM THE DOMESTIC SHEEP[1]

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INTRODUCTION

Although a considerable amount of general information is available concerning the factors that influence the sporulation and viability of oöcysts of sheep coccidia, more specific knowledge is needed for a logical approach to the problem of control of coccidiosis through prevention.

Most of the available facts concerning the behavior of oöcysts of ovine coccidia are attributable to the work of Lerche.[2] Other investigators, namely, Douwes,[3] Yakimoff and his colleagues,[4] and Carré,[5] have largely confirmed and expanded the conclusions of Lerche. From the work of these investigators, it is known that the oöcysts never sporulate inside the intestine of the host because of the prevailing anaerobic conditions; that oöcysts discharged from the host in fecal pellets sporulate within 2 to 3 days, provided conditions of temperature, moisture, and oxygen tension are optimum; that excellent conditions for sporulation often occur in the litter of the fold and in protected sites in pasture grass; that putrefaction and drying are destructive to oöcysts, whereas cold retards the rate of sporulation; and that infection of a new host takes place through ingestion of feed and drink contaminated with fecal material containing sporulated oöcysts.

The general nature of these conclusions may be attributed to lack of detailed experimental work in which all factors have been rigidly controlled. A precise knowledge of conditions favorable or inimical to sporulation is desirable in order to facilitate the recognition and elimination of specific locations in pastures and yards that are favorable to the sporulation and preservation of oöcysts. It is also of practical importance to know how long sporulated or unsporulated oöcysts will retain their viability under given conditions. Conclusions derived from data on sporulation and viability experiments constitute the best basis for formulating measures for the prevention and control of coccidiosis.

The present study was undertaken in order to define more accurately the conditions that favor, retard, or prevent sporulation and destroy or preserve viability of the oöcysts. Although the generalizations presented probably apply equally to all species of ovine coccidia, the data were obtained entirely from experiments on oöcysts of *Eimeria arloingi* Marotel, which occur in greater abundance and frequency

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than those of other species from sheep. An attempt was made in the experimental procedures to duplicate conditions that actually exist in the fold and the pasture in order to make the results directly applicable to conditions existing in the natural environment.

MATERIALS AND METHODS

Freshly discharged oöcysts of *Eimeria arloingi* were obtained from fecal pellets from several 4- to 8-week-old lambs kept near the laboratory of the Zoological Division of the Bureau of Animal Industry, at Beltsville, Md., where this investigation was conducted. Almost daily fecal examinations were made on a total of nine lambs, four during April 1937, and five late in March and early in April 1938. The lambs, with their mothers, were kept in an enclosure 50 feet long by 20 feet wide, and their milk diet was supplemented with water, grain, and hay. Tap water was supplied in a tub, the grain was fed in an open trough upon the ground, and the hay was scattered directly upon the ground. The lambs and ewes had access to the same feed and bedding straw, thereby providing excellent opportunities for the transmission of the infection to lambs from adult animals, the latter being latent carriers.

Few or no oöcysts were discharged by the lambs during the first 3 or 4 weeks after birth, but during the succeeding several weeks enormous numbers were passed. During this period of high oöcyst discharge there was usually a significantly high peak lasting several days during the fourth or fifth week after birth, followed by a lower grade, fluctuating discharge for the next few weeks. Fecal samples were obtained by isolating lambs inside individual, plank-floored pens until pellets were dropped. After release of the animals and collection of samples, each pen was carefully swept, locked, and dried until the next day. Fecal specimens containing oöcysts in sufficient numbers for sporulation experiments could be obtained at any time during the period of high oöcyst discharge.

In the fold and the pasture, discharged fecal pellets containing oöcysts may be subjected to the following conditions: (1) They may fall into running water, which washes the oöcysts relatively free from contaminating organic debris; (2) they may fall into stagnant water and form a layer of putrefying organic sediment on the bottom, in which the oöcysts are lodged; (3) they may fall upon and percolate into litter straw, hay, grass, or grain in feed boxes, where the oöcysts find favorable conditions of moisture for the preservation of viability for indefinite periods; or (4) they may fall in open places exposed directly to the drying action of sunshine and wind. The fecal pellets are exposed to temperature fluctuations, as well as to these natural environmental conditions. In laboratory tests, these possible environments for oöcysts were duplicated as nearly as possible, and each preparation was subjected to several temperatures within the range of those occurring in the natural environment.

To obtain oöcysts free from contaminating debris for sporulation tests in clean water, fresh fecal pellets were crushed in a mortar, mixed with water, and the mixture washed with more water through a 30-mesh sieve into glass containers for settling. After at least an hour of sedimentation, the supernatant fluid was poured off and 1-cc. lots of the sediment were thoroughly mixed with 14-cc. lots of 35-percent sugar solution in 15-cc. centrifuge tubes, which were then allowed to stand for another hour to permit flotation of the oöcysts.

By lifting off the center of the surface film of each tube with a wire loop, the oöcysts were removed from the tubes relatively free from fecal debris. These loopfuls containing oöcysts were placed upon the bottom of the spherical pits of 25- by 75-mm. culture slides, and clean tap water was added to fill the depressions. Oöcysts soon settled to the bottom of the pits and lay beneath about 2 mm. of clean water, with little contaminating debris to obstruct vision or cause putrefaction. The slides were labeled, placed inside moist chambers made by adding water to the floors of Petri dishes, covered, incubated at the desired temperatures, and removed at intervals to determine evidence of sporulation.

A putrefying organic environment was created by placing fecal sediment containing oöcysts in open dishes to depths of about 15 mm., and keeping it barely covered with water by periodic additions during incubation at different temperatures. At room and incubator temperatures it was also necessary to place preparations inside improvised moist chambers to prevent drying. To check for evidence of sporulation, quantities of the sediment were mixed with sugar solution and the floated oöcysts were transferred to slides with a wire loop and observed.

The ability of oöcysts to sporulate inside pellets in which the natural amount of fecal moisture is retained was tested by placing fresh pellets upon a layer of soaked filter paper inside closed Petri dishes and incubating at the desired temperatures. The wet paper insured a saturated atmosphere within the dish, thus preventing loss of fecal moisture. At intervals, oöcysts were removed from the pellets by the flotation method described and examined for evidence of sporulation.

To determine the ability of oöcysts to sporulate inside feces dried in air, fresh pellets were placed in open dishes, stored at the desired temperatures, and the oöcysts were removed at intervals for observation by the flotation method.

In preparations in which little or no sporulation occurred, viability tests were conducted at intervals to determine the longevity of oöcysts under given conditions. The criterion for viability was the ability of the oöcysts to develop normal sporocysts after periods in the unsporulated condition, when the protoplasm is in the form of a spherical sporont. The viability test consisted in isolation of oöcysts in clean water on culture slides and storage for at least 3 days at room temperature. If no sporulation occurred within that time, the oöcysts were considered as dead, since maximum normal sporulation of oöcysts of *Eimeria arloingi* takes place within 48 hours after removal into clean water at room temperature.

In the results reported in this paper, oöcysts were classified as follows: Those in which there was no sign of protoplasmic segmentation, as oöcysts with undivided sporont; those containing four-lobed sporonts, four spheres, pyramids, or oval sporoblasts, as oöcysts with the intermediate stages; those in which the protoplasm had split into two to eight or even more fragments of unequal size, as oöcysts showing abnormal segmentation; and those containing four refractile equal-sized, roughly spindle-shaped sporocysts, as oöcysts with complete sporocysts.

The longevity of oöcysts in the sporulated condition obviously cannot be determined by the method described in this paper. The

criterion for viability of sporulated oöcysts is the production of infection in a susceptible host, which is beyond the scope of the present study. It is believed, however, that the conclusions derived from these viability experiments with unsporulated oöcysts can be applied as well to those in the sporulated state, with the advantage in favor of the latter as a result of an additional protective shell about each sporocyst.

SPORULATION AND VIABILITY OF OÖCYSTS OF EIMERIA ARLOINGI IN SHALLOW TAP-WATER CULTURES

Oöcysts in shallow tap-water cultures kept at near-freezing temperature (0°–5° C.) sporulated slowly, 2 to 3 weeks elapsing before a significant amount of sporulation was noted (table 1). At room temperature (20°–25°) segmentation was rapid and normal, maximum sporulation occurring within 48 hours. Since these cultures gave uniformly higher percentages of sporulation than any others in the series, the conditions were considered to be optimum for sporulation and became the basis for viability tests. Oöcysts in cultures kept at 32° segmented at an accelerated rate but in an abnormal manner. There were evidences of protoplasmic splitting in more than half of them at the end of 24 hours. At 72 hours, which is an interval sufficient for the production of four equal-sized spores in each oöcyst in cultures kept at room temperature, the protoplasm of most of the oöcysts had segmented into two to eight, occasionally more, fragments of unequal size and shape. There was no sign of sporulation in oöcysts kept at 40° C. for as long as 10 days.

TABLE 1.—*Sporulation and viability of oöcysts of Eimeria arloingi beneath 2 mm. of clean tap water*

Experiment No.	Temperature of preparation during sporulation test	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test					Results of viability test [1]			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts showing abnormal segmentation	Oöcysts with complete sporocysts	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	° C.			*Days*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*Pct.*	*Pct.*	*Pct.*
1	0–5	1	Apr. 13, 1937	7	40	100	0	0	0	------	------	------	------
				13	111	59	23	0	18	------	------	------	------
		2	Mar. 30, 1938	12	70	100	0	0	0	------	------	------	------
				16	66	76	21	0	3	------	------	------	------
				23	56	7	63	0	30	------	------	------	------
2	20–25	1	Apr. 19, 1937	5	694	4	0	0	96	------	------	------	------
				Hrs.									
		2	May 27, 1937	26	121	50	24	0	26	------	------	------	------
				47	129	6	6	0	88	------	------	------	------
		3	Mar. 30, 1938	24	90	58	42	0	0	------	------	------	------
				30	95	19	38	0	43	------	------	------	------
				48	88	3	3	0	94	------	------	------	------
3	32	1	Apr. 5, 1938	24	108	47	7	26	20	------	------	------	------
				41	107	18	7	54	21	------	------	------	------
				72	126	10	2	73	15	------	------	------	------
				Days									
4	40	1	Mar. 30, 1938	1	100	100	0	0	0	79	34	63	3
		2	Apr. 1, 1938	3	300	100	0	0	0	274	60	40	0
		3	Apr. 5, 1938	10	100	100	0	0	0	100	100	0	0

[1] Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The results of the viability test, also given in table 1, showed that practically all the oöcysts kept at 40° C. in shallow tap-water cultures were killed by exposure for 2 days to that temperature and that all were killed in cultures exposed 3 and 10 days.

Failure to sporulate normally or at all at the higher temperatures is attributed to insufficiency of oxygen, since the oxygen-holding capacity of water diminishes with increase in temperature.

IN PUTREFYING FECAL SEDIMENT

At near-freezing temperature (0°–5° C.), all oöcysts in fecal sediment covered with shallow water remained unsporulated after 10 months' exposure (table 2); at room temperature (20°–25°), all were unsporulated after 1 year; and at 40°, there was no sporulation in 17 days. The protoplasm in these oöcysts retained the form of spherical sporonts, which gradually diminished in size and showed progressive disintegration as preparations aged.

TABLE 2.—*Sporulation and viability of oöcysts of Eimeria arloingi in fecal sediment covered with shallow water*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test		Results of viability test [1]			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	°C.			Days	Number	Percent	Number	Percent	Percent	Percent
1	0–5	1	May 26, 1937	50	100	100	200	4	0	96
				201	100	100	223	18	0	82
				314	200	100	123	19	62	19
		2	Sept. 22, 1937	113	50	100	50	4	0	96
2	20–25	1	Apr. 12, 1937	308	100	100	100	0	100	0
		2	Apr. 16, 1937	33	89	100	40	48	0	52
		3	May 25, 1937	127	100	100	38	100	0	0
		4	Apr. 1, 1938	19	100	100	54	6	0	94
		5	Apr. 11, 1938	7	100	100	100	4	0	96
3	40	1	May 25, 1937	17	212	100	156	100	0	0
		2	Mar. 31, 1938	4	100	100	46	78	22	0

[1] Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The viability test showed the approximate point at which death of the protoplasm took place. Oöcysts kept in fecal sediment at near-freezing temperature lost viability slowly and gradually, as seen in table 2. There was no perceptible loss in ability to sporulate in nearly 4 months, after which oöcysts gradually died until only about one-fifth of them were viable at the end of 10 months. At room temperature the loss of viability was more rapid. Approximately half of the oöcysts remained viable at 1 month, whereas all were killed within 4 months. All oöcysts in preparations incubated at 40° C. were killed within 4 days.

The absence of sporulation in the oöcysts is attributed to the oxygen insufficiency resulting from putrefaction rather than to toxic products of the process. The fact that about 20 percent of these unsporulated oöcysts were still viable after 10 months at near-freezing temperature indicates that oöcysts may accumulate in wet situations in the outside environment and survive for many months during colder parts of the year and that they retain the ability to sporulate

and become infective to susceptible hosts when appropriate conditions are restored. This ability of oöcysts to live for long periods in wet situations in colder weather and the marked lethal influence of high temperatures suggest the advisability of selecting sunny, well-drained terrain for sheep lots and pasture and of eliminating sites that favor the accumulation of waste water.

INSIDE FECAL PELLETS IN WHICH NATURAL MOISTURE WAS PRESERVED

At near-freezing temperature (0°–5° C.), oöcysts inside fecal pellets in which natural moisture was preserved sporulated slowly, only about one-fifth of them showing signs of segmentation within 10 months (table 3). Sporulation was rapid at room temperature (20°–25°), only 3 days being required for the majority of the oöcysts to develop mature sporocysts. There was no sign of sporulation within 4 days in preparations incubated at 40°.

TABLE 3.—*Sporulation and viability of oöcysts of Eimeria arloingi inside fecal pellets in which natural moisture was preserved*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test				Results of viability test [1]			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts with complete sporocysts	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	°C.			*Days*	*Number*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
1	0–5	1	May 26, 1937	10	100	100	0	0	106	6	0	94
				126	117	86	12	2	100	7	0	93
		2	Apr. 1, 1938	314	173	82	15	3	155	19	57	24
				10	100	100	0	0				
2	20–25	1	Apr. 19, 1937	3	577	29	11	60				
				9	744	20	0	80				
				36	103	3	0	97				
		2	Mar. 31, 1938	1	163	92	8	0				
				2	127	45	13	42				
				4	109	46	4	50				
		3	Apr. 11, 1938	3	98	4	1	95				
				7	100	5	0	95				
		4	do	3	100	9	1	90				
				7	100	0	0	100				
3	40	1	Mar. 31, 1938	4	100	100	0	0	100	98	2	0

[1] Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The results of the viability test (table 3) showed that there was no appreciable loss of ability to sporulate in oöcysts inside natural pellets kept for 4 months at near freezing temperature, but during the next 6 months three-fourths of them lost viability. At 40° C., oöcysts inside the pellets were rapidly killed, being unable to sporulate after an exposure of 4 days.

These observations suggest the desirability of frequent changes of bedding straw and also of feeding from racks and elevated troughs rather than off the ground in order to eliminate environmental conditions favorable for the preservation of the natural moisture in fecal pellets and to reduce the chances of feed being contaminated with sporulated oöcysts.

INSIDE FECAL PELLETS DRIED IN AIR

Inside fecal pellets dried at room temperature (20°–25° C.), sporulation occurred before total desiccation. A majority of the oöcysts sporulated within 3 days (table 4) before the oöcyst walls were so extensively wrinkled and blackened that it was impossible to distinguish the contents. After 2 days of drying at 40°, oöcyst walls were extremely wrinkled, distorted, and blackened, but one-sixth of the oöcysts contained what appeared to be normal sporocysts.

TABLE 4.—*Sporulation of oöcysts of Eimeria arloingi inside fecal pellets dried in air*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of test	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts in which wrinkling hid contents	Oöcysts with recognizable sporocysts
	° C.			*Days*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
1	20–25	1	Apr. 5, 1938	1	100	100	0	0	0
				2	93	3	5	38	54
				6	100	0	0	100	0
		2	Apr. 11, 1938	3	67	10	0	10	80
				7	54	0	0	93	7
2	40	1	Apr. 5, 1938	2	104	39	2	43	16
				6	100	0	0	100	0

The data of these experiments indicate that drying is probably fatal to oöcysts within several days or a few weeks as a result of the loss of water necessary for the vital activities of protoplasm. This observation again emphasized the importance of dry, well-drained land for sheep lots and pasture.

SUMMARY

The results of sporulation and viability experiments on oöcysts of *Eimeria arloingi* from domestic sheep are presented. These experiments demonstrated several facts of practical importance in the control of ovine coccidiosis.

Oöcysts covered with 2 mm. of clean water sporulated slowly at near-freezing temperature (0°–5° C.) and normally and rapidly at room temperature (20°–25°). At 32° sporulation was accelerated but segmentation was abnormal, and at 40° the oöcysts failed to show signs of sporulation. The viability test showed that the unsporulated oöcysts in the cultures incubated at 40° were killed when exposed for 3 days. Abnormal segmentation and failure to sporulate at the higher temperatures were apparently due to insufficiency of oxygen.

There was no indication of sporulation at near-freezing, room, or incubator temperatures in oöcysts kept in fecal sediment covered with a shallow layer of water. Failure to sporulate under these conditions was attributed to lack of oxygen resulting from putrefaction. About 20 percent of the unsporulated oöcysts were still viable after 10 months at near-freezing temperature, indicating that oöcysts may accumulate in wet situations in the outside environment and survive for many months during the colder parts of the year, and that they retain the ability to sporulate and become infective to susceptible hosts when appropriate conditions are restored.

Oöcysts inside fecal pellets in which moisture was preserved showed little sporulation at near-freezing temperature, abundant and rapid sporulation at room temperature, and failure to sporulate at 40° C. These observations indicate that simple, sanitary measures in lamb raising, such as frequent changes of bedding straw and feeding from elevated racks and troughs, would greatly reduce the chances of contamination of the feed with resultant infection.

When fecal pellets were dried in air at room temperature and at 40° C., many of the contained oöcysts sporulated before desiccation had produced such extensive wrinkling and shrinkage that recognition of contents was impossible. It was concluded that drying was probably fatal to oöcysts within several days or a few weeks, emphasizing the importance of dry, well-drained land for sheep lots and pasture.

THE NATURE OF GROWTH SUBSTANCE ORIGINATING IN CROWN GALL TISSUE [1]

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INTRODUCTION

The presence of growth substance in more than the normal concentration (*10*)[2] has been demonstrated in cultures of the crown gall organism, *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. (*1, 2, 9, 10, 11*) and also in recently inoculated gall tissue. The growth substances obtained from cultures of micro-organisms have been identified in a number of instances as beta-indole-acetic acid (*1, 7, 9, 14*). Early reports identified growth substances from the higher plants as auxin *a* and *b* (*4, 5, 6*). More recently the presence of beta-indole-acetic acid in higher plants has been reported (*8*). It is desirable to know the nature of the growth substance in crown gall for various reasons; e. g., should the growth substance normally produced by the host plant prove to be different from that produced by the crown gall organism in culture, it would be possible to determine whether the growth substance present in the gall tissue is produced by the bacteria or by the host cells. Consequently, the identification of the growth substances obtained from tomato gall tissue, from tomato foliage, and from crown gall culture was undertaken.

METHODS

The method chosen was that of Kögl and his associates (*6*). Beta-indole-acetic acid is stable in basic solution and unstable in acid solution; auxin *a* is stable in acid solution and unstable in basic solution, and auxin *b* is unstable in both acid and basic solution. Thus, it is possible to classify these growth substances on this basis.

The gall tissue was obtained from inoculations on stems of tomato (*Lycopersicum esculentum* Mill.). Before inoculation the plants were decapitated and the leaves and axillary buds were removed from the stems as far down as the inoculations extended. This was done to prevent the growth substance that is normally produced in these parts from entering the gall tissue. Six weeks after inoculation the galls were harvested and prepared for study. Some were extracted fresh and some were frozen and dried while frozen in the lyophile apparatus (*3*). An ether extract made of denuded, uninoculated stems indicated that growth substance was present in a concentration equal to 0.007 gamma of beta-indole-acetic acid per denuded stem weighing 0.4 gm. This is about one-tenth as much as was found in parallel material from inoculated stems which, however, weighed about 4 gm. Thus, the growth substance present in 1 gm.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 538.

(wet weight) of tissue was not very different in either case, being about 0.0018 gamma. Doubtless higher yields of growth substance can be secured with improvements in methods of extraction.

Growth substance from the above-described material, from tomato foliage, from crown gall culture, and from corn meal, as well as beta-indole-acetic acid, were all tested for stability toward acid and base. Beta-indole-acetic acid and corn-meal extract (auxin *a* and *b*) were included as checks on the method. The tomato foliage and culture extracts were included for comparison with the gall extract.

Fresh tissue for growth substance was extracted according to Van Overbeek's method (*12*). One kilogram of fresh sliced tissue was immersed in 2,000 cc. of peroxide-free ether and left overnight at 4° C. The following morning the ether extract was placed on a steam bath. The residues in this case and in others mentioned below were taken up in 5 cc. of distilled water. Dried tissue and corn meal were similarly extracted at the rate of 50 gm. to 500 cc. of ether. Extractions of crown gall cultures grown in peptone broth were made 3 months after seeding with 500 cc. of ether to each 250 cc. of culture.

Following, as far as practicable, the procedure of Kögl et al., the sensitivity toward acid and base was tested. One cubic centimeter of growth-substance solution was placed in each of three vials. To the first was added ½ cc. N/2 HCl; to the second, ½ cc. N/2 KOH; and to the third ½ cc. distilled water and one drop of saturated phosphate buffer (pH 7.0). The three vials were loosely covered and steamed for 3 hours in the Arnold steamer. After removal from the steamer they were neutralized. One drop of saturated phosphate buffer (pH 7.0) was then added to vials 1 and 2. They were then tested for the presence of active growth substance. One cubic centimeter of untreated growth substance solution was diluted with 1 cc. of distilled water (pH 7.0) and tested along with the treated samples.

Growth substance was estimated by means of Went's standard technique (*15*) as discussed earlier (*11*). The growth-substance concentration of each preparation was expressed as the average negative curvature produced in 12 coleoptiles.

EXPERIMENTAL RESULTS

The results are summarized in tables 1 and 2. These are based on 12 trials involving 6,912 *Avena* coleoptiles. Not reported are results of 8 preliminary trials involving 576 coleoptiles. These trials were made in the course of determining the most usable concentration of acid and base and the most desirable time of treatment. While these results were in complete agreement with those reported, they were omitted for the sake of uniformity and brevity.

The growth substances from the gall tissue, tomato foliage, and crown gall culture were all much more stable in basic solution than in acid solution. This suggests that some substance comparable in certain reactions to beta-indole-acetic acid was present in the ether extracts of these materials. These same preparations showed some reduction in activity below that of the untreated samples, which might have been slightly greater than indicated here, because the untreated samples gave curvatures beyond the maximum quantitative angle. However, other trials within the quantitative range indicate that this reduction has little if any effect on the conclusions drawn. Following treatment in basic solution, there was much less or no reduction in

activity below that of the sample treated in neutral solution. Consequently, there is little or no evidence in these data that any growth substance unrelated to beta-indole-acetic acid is involved in the crown gall material studied.

TABLE 1.—*Average negative curvatures of Avena coleoptiles obtained with growth substances from crown gall and several other sources before and following heat treatments in acid, basic, and neutral solutions* [1]

Source of growth substance	Average negative curvature of *Avena* coleoptiles following the treatments indicated			
	Acid	Basic	Neutral	Untreated
	Degrees	*Degrees*	*Degrees*	*Degrees*
Beta-indole-acetic acid	4	15	20	24
Do	2	12	20	25
Corn meal	18	13	24	23
Do	20	12	20	24
Fresh tomato gall tissue	3	16	12	20
Do	1	15	16	16
Lyophilized tomato gall tissue	0	18	21	18
Do	1	11	13	13
Fresh tomato foliage	7	17	19	24
Do	2	13	11	21
Crown gall culture	12	24	20	26
Do	1	15	8	13

[1] Comparison can be made horizontally, between treatments of the same material, but should not be made vertically between different materials.

TABLE 2.—*Reduction in activity of growth substances from crown gall tissue and several other sources following heat treatment in acid, basic, and neutral solution*

Source of growth substance	Reduction in activity following treatments indicated in percentage of untreated sample		
	Acid	Basic	Neutral
	Percent	*Percent*	*Percent*
Beta-indole-acetic acid	88	45	15
Corn meal	20	45	7
Fresh tomato gall tissue	88	11	20
Lyophilized tomato gall tissue	94	6	0
Fresh tomato foliage	81	32	32
Crown gall culture	72	3	31

The method distinguished between beta-indole-acetic acid and auxins *a* and *b* in trials made with beta-indole-acetic acid and an ether extract of corn meal. The indolic acid was much less stable in acid solution than in basic solution, although in the basic solution there was some reduction in activity below that in neutral solution. The auxin was highly stable in neutral solution, but was only moderately stable in acid solution and somewhat less so in basic solution. This suggests the presence of both auxin *a* and auxin *b*, the figures obtained in these trials indicating a mixture of about equal amounts of the two types. The activity following basic treatment is attributed to incomplete inactivation of auxin *b* rather than to the presence of beta-indole-acetic acid because the sum of the curvatures obtained after acid and after basic treatment far exceeds the curvature obtained with the untreated sample.

The growth substance obtained from the foliage of the normal plant proved to be indistinguishable, on the basis of these tests, from that in the crown gall culture. Thus it is not possible at present to conclude whether the bacteria or the host cells supply the growth substance in crown gall tissue. While these tests with crude extracts are not nearly so reliable as diffusion experiments might be, they suggest (1) that the active substance in gall and stem are similar; and (2) that this substance resembles beta-indole-acetic acid in its sensitivity to acid and alkali. Lefèvre (*8*) determined the phenyl or indole compounds in expressed juice, but these may not be active in causing growth.

While it appears that the same type of growth substance occurs in crown gall culture and crown gall tissue, it seems wise to reserve judgment about beta-indole-acetic acid being the major cause of gall formation. Among the reasons are: (1) The amount of growth substance detected in crown gall tissue is a very small fraction of that required to produce a similar proliferation with artficial application (*10*); (2) substances diffusing from inoculations made with virulent crown gall bacteria stimulate development of galls by inoculation with attenuated crown gall bacteria, while even strong applications of beta-indole-acetic acid do not (*10*); and (3) responses of a number of plants to crown gall bacteria do not parallel their reactions to applications of beta-indole-acetic acid (*13*).

SUMMARY

Growth substances obtained by ether extraction from tomato crown gall tissue, tomato foliage, and crown gall culture on peptone broth all appeared to contain either beta-indole-acetic acid or material similar in its sensitivity to acid and alkali. Identification was based on the stability of a crude extract in hot acid and basic solutions. No evidence was obtained of the presence of auxin *a* or *b* in these extracts. The growth substance measured might have come either from the plant or from the bacteria.

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INFLUENCE OF BORON ON FLOWER-BUD DEVELOPMENT IN COTTON[1]

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INTRODUCTION

Although the role of boron in plant nutrition has received a great deal of attention in the past few years most investigators have emphasized the relation of this element to the vegetative growth of plants or to imperfections in the fruit. Brenchley and Warington,[2] Johnston and Fisher,[3] Shive,[4] Eaton,[5] and others have called attention to the influence of boron on fruiting but have offered no evidence to show that any particular phase of the fruiting cycle was specifically concerned.

The results reported in this paper emphasize the phase of the reproductive cycle of cotton in which the boron supply may be a limiting factor.

MATERIALS AND METHODS

Jars and special covers of pyrex glass used in other water-culture studies [6] were employed in these experiments. Investigators in this field have generally avoided borosilicate glass, but experience has shown that pyrex glass does not supply sufficient boron to the young cotton seedlings in midsummer to protect them from severe boron deficiency even in the first week of growth (fig. 1).

Salts for the nutrient solutions were of the same lot used in other trace-element studies and for the 1937 cultures chemically pure salts were recrystallized three times from water redistilled from pyrex stills. For the 1938 cultures two additional recrystallizations were made from water double-distilled from pyrex stills.

The basal solution had the following composition:

	Mole
$Ca(NO_3)_2$	0.0030
$MgSO_4$	.0020
KH_2PO_4	.0010

To all cultures was added 0.1 p. p. m. Mn as $MnSO_4.2H_2O$. Iron as ferric tartrate was added as needed. Manganese and boron were the only so-called trace elements added to the culture solutions in 1937 or at the beginning of the 1938 work. However, the 1937 plant leaves never had a satisfactory green color and after the same faint and persistent chlorosis developed in the 1938 cultures, copper as

[1] Received for publication March 24, 1939, Paper No. 62 of the Journal Series of the Georgia Experiment Station.

[2] BRENCHLEY, WINIFRED E., and WARINGTON, KATHERINE. THE ROLE OF BORON IN THE GROWTH OF PLANTS. Ann. Bot. [London] 41: 167–187, illus. 1927.

[3] JOHNSTON, EARL S., and FISHER, PAUL L. THE ESSENTIAL NATURE OF BORON TO THE GROWTH AND FRUITING OF THE TOMATO. Plant Physiol. 5: 387–392, illus. 1930.

[4] SHIVE, JOHN W. THE ADEQUACY OF THE BORON AND MANGANESE CONTENT OF NATURAL NITRATE OF SODA TO SUPPORT PLANT GROWTH IN SAND CULTURE. N. J. Agr. Expt. Sta. Bul. 603, 36 pp., illus. 1936.

[5] EATON, FRANK M. BORON REQUIREMENTS OF COTTON. Soil Sci. 34: 301–305. 1932.

[6] GEORGIA EXPERIMENT STATION. MANGANESE REQUIREMENTS OF COTTON. Ga. Expt. Sta. Ann. Rpt. (1937–38) 50: 62–63. 1938.

FIGURE 1.—*A*, Cotton seedlings after growing 37 days in a nutrient solution, without added boron, in a pyrex jar; *B*, plant from group in *A* showing failure of terminal bud development.

$CuSO_4.5H_2O$ and zinc as $ZnCl_2$ at the rate of 0.01 p. p. m. and 0.1 p. p. m., respectively, were added to each jar on July 20 and at each renewal thereafter. The color of the leaves of these plants after these additions was satisfactory. Boron was added as boric acid. The stock solutions and distilled water used in preparing these nutrients were stored and handled in pyrex glass.

The solutions were renewed June 29, July 16, August 5, 19, 27, 1937, and June 15, 27, July 14, 25, and August 8, 19, 1938. In the later stages of growth, the nitrogen was almost completely absorbed within 3 or 4 days after the solutions were renewed, and consequently growth was limited by insufficient nutrient supply.

The delinted seed of the Durango variety which was used in these cultures contained 23 p. p. m. of boron or about 3 gamma per seed. Fifteen seedlings were started in each jar and they were thinned to five after about 2 weeks and to two after about 5 weeks. After the beginning of the fruiting stage in 1937, the flower buds, or squares, were tagged with white tags and counted once each week. However, in the late-growth stages it was found that many buds were formed and shed within 1 week and that no accurate record of total buds formed could be obtained for the 1937 crop. In the 1938 studies

the buds were tagged and counted twice each week. Every morning white tags were removed from newly opened blooms and replaced by colored tags. In the 1938 cultures the colored tags were dated.

EXPERIMENTAL DATA

In the 1937 cultures there was no noticeable difference in the general appearance of the plants at the different boron levels for the first 8 or 9 weeks. Abscission of flower buds and scarcity of blooms, however, were noticeable within the first 9 weeks on plants at the 0.1-p. p. m. boron level. As the growing period advanced, the young buds were shed when they were much smaller and, finally, there was some irregularity in leaf development in the growing tops of these plants, indicating a deficiency of boron for the young growing points. At this stage the young flower buds blackened and died in a manner comparable to that seen in terminal buds in cases of severe boron deficiency.

TABLE 1.—*Influence of boron supply upon growth and flowering of cotton planted June 15 and harvested Sept. 11, 1937*

[Average of four plants]

Boron added (p. p. m.)	Height of plants	Green weight of plants	Flower buds Sept. 11	Blooms
	Centimeters	*Grams*	*Number*	*Number*
0.1	163.7	1,076.0	53.1	10.7
1.0	172.5	1,257.0	46.7	53.2
5.0	148.7	1,199.0	29.7	45

As the data of table 1 show, there was no decided difference in the vegetative growth of the plants at the different boron levels, but the 0.1-p. p. m. boron plants produced very few blooms. Most of the green-weight difference was due to the young bolls on the plants at the higher boron levels.

The experiment was repeated on a larger scale in 1938. Again there was little difference in the appearance of the plants at the two boron levels for the first 8 weeks. The flower buds on the plants at the lower boron level developed to a fair size and then most of them became chlorotic, the bracts flared open, and they dropped. As the growing period advanced, the flower buds abscissed at smaller sizes so that during the last 10 days of the study they darkened and dropped when they were so small that they were scarcely recognizable, and it is probable that the recorded figure for total flower buds for this series is low in spite of the fact that they were tagged twice each week. About August 15 the branches near the tops of this group of plants became very brittle, developed short internodes, and the leaf buds tended to darken and show other irregularities in development usually associated with boron deficiency. This development is illustrated in figure 2. As table 2 shows, the results are in general agreement with those of 1937.

In this case the mean difference in flowers per plant, 40.4, with a pooled standard deviation of 10, is highly significant.

FIGURE 2.—Low-boron plant (0.1-p. p. m. group, Table 2), August 28, 1938, showing irregularity of leaf development near top.

TABLE 2.—*Influence of boron supply upon growth and flowering of cotton planted June 3 and harvested Aug. 28, 1938*

[Average of 14 plants]

Boron added (p. p. m.)	Height of plants	Green weight of plants	Flower buds Aug. 28	Flower buds shed	Total flower buds	Blooms
	Centimeters	*Grams*	*Number*	*Number*	*Number*	*Number*
0.1	155.1	1,149.0	54.6	98	162.9	10.2
1.0	182.0	1,303.0	62.1	40	153.2	50.6

DISCUSSION

Since the nutrient concentration was not held constant, these results give little indication of the optimum concentration of boron for growth and fruiting of cotton in water culture. They do emphasize, however, the importance of boron in the flowering of cotton and indicate that an insufficiency of this element may be the cause of unfruitfulness. Although approximately 70 percent of the flowers of the low-boron plants, 1938 series, appeared by August 10, before boron deficiency had become manifest in the vegetative parts of the plants, the abscission of young buds from this group of plants and from those at the same level the previous year was apparently abnormal in the early stages. This observation along with the fact that vegetative growth was not severely checked at any stage in the low-boron series suggests that the concentration of this element necessary for flower-bud development in cotton is higher than that required by the vegetative parts. But the blackening of young flower buds in a manner quite similar to that observed in young leaf buds in cases of severe boron deficiency also suggests that the specific effects of the deficiency upon the tissues involved are probably the same.

From the records of total flower buds per plant (table 2) it may be seen that the boron level which was too low for flower-bud development had no apparent effect on flower-bud initiation.

SUMMARY

Water-culture studies of the boron requirements of cotton brought out the fact that this element is necessary for flower-bud development in this plant, and that flowering may be seriously limited by a supply of boron that is sufficient for fair vegetative growth.

There is no evidence from these results that boron has any relation to flower-bud initiation in cotton.

PHOTOSYNTHETIC STUDIES OF MUTATIONAL BARRENNESS IN THE MONTMORENCY CHERRY[1]

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INTRODUCTION

The reality of bud mutation by means of which unique plant forms arise is no longer seriously doubted. That the mutant's peculiar, not to say abnormal, behavior has a discoverable physiological basis seems altogether likely. And this, be it said, is not to rule out genetics, since no objection need be made to the notion of an underlying genetic condition, enforcing and finding its expression through altered yet controlled physiological processes.

A case in point is that of a bud mutant showing the permanent characteristic of not forming fruit buds and of being always barren. Investigations have shown that an accumulation of carbohydrates in the spurs of spur-bearing trees is necessary for fruit-bud differentiation. The rate of carbohydrate synthesis must exceed the rate of utilization of the carbohydrates in vegetative growth if there is to be an accumulation in the tissues of the spurs. Possibly the defect of this antecedent condition—a rate of photosynthetic activity that is relatively too low—characterizes the perpetually barren mutant. To test the validity of this hypothesis a series of experimental observations was made on a mutant in the Montmorency cherry (*Prunus cerasus* L.).

TREES USED IN THE TESTS

In the beginning, a limb mutant was detected and marked in an orchard of Montmorency trees near South Haven, Mich. The limb was conspicuous for its lack of fruit-bud formation and consequent barrenness. During the season of 1925, after several years of this behavior, buds were taken from it and propagated on mahaleb stock. Six of the trees thus obtained were selected in 1929 and transferred from the nursery to an orchard plot at the Graham Horticultural Experiment Station, Grand Rapids, Mich. The trees were planted in a row, the usual distance apart, and thereafter were given ordinary attention and culture. Upon coming to bearing age, they exhibited the following characteristics, which have remained constant:

Tree 1. Barren, except two main limbs that are normal in fruitfulness.
Tree 2. Wholly barren.
Tree 3. Barren, except one main limb that is somewhat productive.
Tree 4. Like tree 1, except that only one limb is fruitful.
Tree 5. Wholly barren.
Tree 6. Entirely normal in bearing habit and productivity.

In 1934, 5 years after the trees were planted, experiments were begun to determine whether or not the mutational barrenness arose from inadequate synthesis of carbohydrates and a deficiency of their accumulation in the spurs.

[1] Received for publication September 24, 1938. Journal article No. 233 (n. s.), Michigan Agricultural Experiment Station.

ANALYSIS OF SPURS

Samples of spurs were taken at intervals between June 6 and August 3, 1934. Tree 5 (barren) and tree 6 (normal) were used for comparison. Sampling was restricted to the nonbearing spurs of lateral branches on the wood of the season before, and was randomized by the selection of laterals generally around and over the tree.

The sample, consisting of 125 to 200 spurs, was heated immediately at 90° C. for 40 minutes. It was then transferred to an oven held at 67° C. and brought to dryness. Its preparation for analysis was completed by grinding to pass a 60-mesh sieve. Later, in actual readiness for analysis, drying was accomplished at 70° C.

Free reducing substances, starch, and polysaccharides other than starch, were determined. The analytic procedure for each of these was the customary one, involving the Shaffer-Hartmann and Quisumbing-Thomas routine. The results are shown in figure 1.

At Grand Rapids detectable fruit-bud differentiation in the Montmorency cherry begins within the last 2 weeks of July. Doubtless this observable stage is preceded by a period of incipient preparation, the beginning and length of which, if knowable at all, has not been determined. Figure 1 shows that fruit-bud differentiation was preceded by an emphatic rise in free-reducing substances in the spurs, followed by a steep and prolonged decline. A second upward tendency coincides with its visible inception and continues during its progress.

The graphs for polysaccharides other than starch are the inverted image of those for free-reducing substances. As to starch, the content increases continuously, from the beginning for the normal tree and after June 21 for the barren (mutant) tree.

A general comparison of the two trees shows that the normal one runs higher in free-reducing substances and starch and lower in polysaccharides other than starch. This indicates both the maintenance of a more abundant supply of elemental carbohydrates in the normal tree and their sustained accumulation, in the form of a higher content of starch, in its spurs. The latter finding is the more remarkable because the tree supported a full crop of fruit until the first week in July, the time of harvest.

Amino nitrogen was determined for the same samples, the Van Slyke apparatus and technique being employed for its estimation. The determinations are presented graphically in figure 2.

Amino acids, the simpler elements for the synthesis of proteins, increase in the spurs in the early part of the season, and then decline (fig. 2). The falling off starts later in the barren tree (July 6) than in the normal. Thereafter it is continuous in this tree, whereas in the normal tree it ceases about midway in the seasonal range, and thenceforward, just before and through the period of fruit-bud formation, holds at this higher level.

All in all, the results suggest a relatively low rate for photosynthetic activity, and for the production and accumulation of starch, both prior and subsequent to fruit-bud formation, in tree 5, the strictly mutational tree. However, the significance of such analyses, especially for samples infrequently taken, should not be given too much weight. Their bearing upon the rate of photosynthetic activity and manufacture is not as close as is required for conclusive interpretation, and

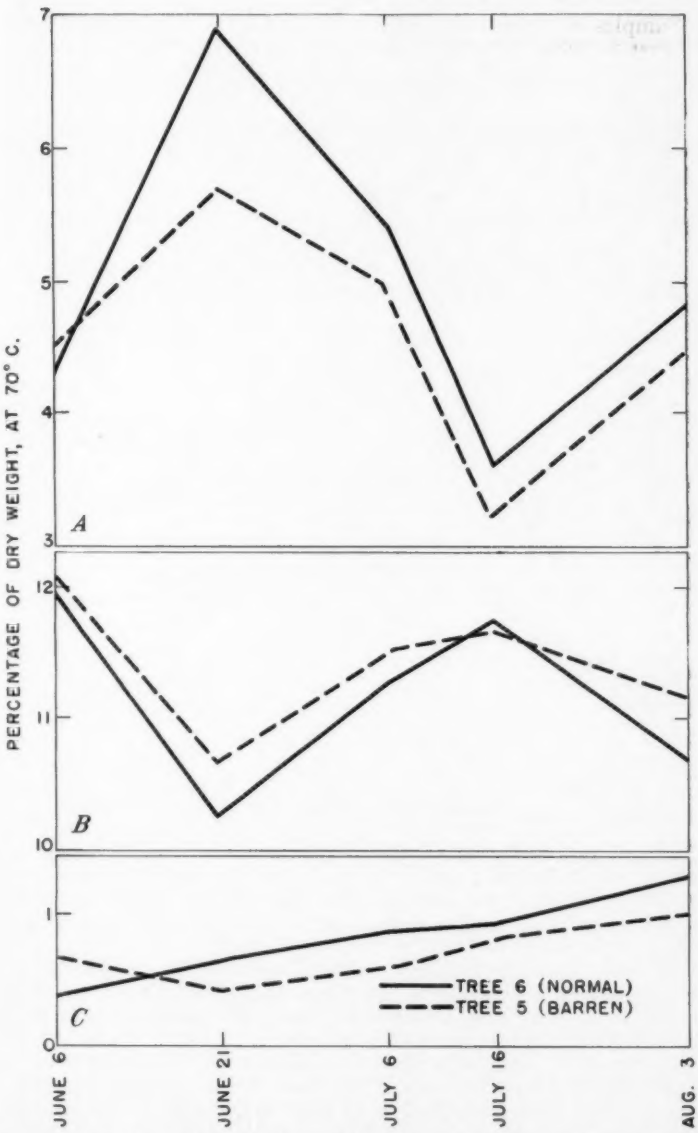

FIGURE 1.—Free reducing substances (*A*), polysaccharides other than starch (*B*), and starch (*C*) in spurs from a barren and from a normal tree between June 6 and August 3, 1934.

they should be checked by experimental procedures that are more direct and at the same time better adapted for securing data essential to the calculation of a rate for a physiological process.

PHOTOSYNTHESIS STUDIES

Photosynthesis determinations were made during the season of 1936, usually at regular intervals, on samples taken every 2 hours on clear days during a period of 24 hours.

The samples were obtained by the leaf-punch method, devised by Ganong. When refined by the familiar corrections for translocation and respiration, and changes in the weight of ash, as it was in this work, this method is satisfactory for use in the orchard and for taking many samples at random over a tree or any large part of a tree.

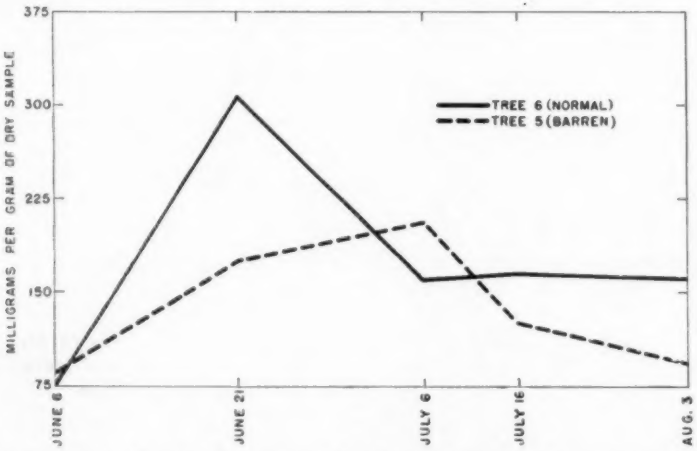

FIGURE 2.—Amino nitrogen in spurs taken from a barren and from a normal tree between June 6 and August 3, 1934.

Each sample consisted of 30 disks—one each from the leaves of spurs in the middle sections of laterals taken generally over the tree or the portion of the tree involved in the comparison. The Ganong leaf punch cuts a disk 1 cm.^{2} in area. Hence, a sample was equivalent to 30 cm.^{2} of leaf surface.

The trees were cultured and sprayed alike. The spray material, when present, was wiped from the leaves selected for use, at the beginning of the 24-hour sampling period. Its uneven distribution on the leaves necessitated this precaution.

The first three samplings (shown in table 1) were with leaves of the spurs of laterals on 1934 wood. In the first two of these three samplings fruiting spurs were chosen on tree 6 and on the fruitful parts of trees 1 and 3 for comparison with barren spurs on tree 5 and the barren parts of trees 1 and 3. Thereafter, beginning July 25, nonfruitful spurs were selected exclusively throughout, these on the laterals of 1935 wood. The results are given in table 1.

In table 1, the ten 24-hour series are divided in half by the first row of totals and corresponding rates. The upper half, July 8–9 to August 8–9, covers approximately the period from the picking of the fruit, when present, to the conclusion of the process of fruit-bud formation. The lower half, August 15–16 to September 17–18, covers the time from fruit-bud formation to the onset of senility in the leaves.

TABLE 1.—*Amount and rate of photosynthate production for spur leaves from barren and fruitful trees or parts of trees, 1936*

Date	Total photosynthate in 24 hours per square meter of leaf area				Photosynthate produced per hour per square meter of leaf area			
	Trees 1 and 3		Tree 5 (barren)	Tree 6 (normal)	Trees 1 and 3		Tree 5 (barren)	Tree 6 (normal)
	Barren parts	Fruitful limbs			Barren parts	Fruitful limbs		
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
July 8–9	−3.133	−2.533	−3.633	+1.667	−0.131	−0.106	−0.151	+0.069
July 17–19	−2.633	+2.700	−1.699	+.366	−.110	+.113	−.071	+.015
July 25–26	+3.799	+9.332	−.167	+2.833	+.158	+.389	−.007	+.118
Aug. 1–2	−5.099	+1.367	−2.933	−4.633	−.213	+.057	−.122	−.193
Aug. 8–9	−.933	−2.566	+1.467	+5.900	−.039	−.107	+.061	+.246
Total	−7.999	+8.300	−6.965	+6.133	−.067	+.069	−.058	+.051
Aug. 15–16	−.967	−6.767	+2.100	+.467	−.040	−.282	+.087	+.020
Aug. 22–23	+1.933	+1.567	+8.133	−1.733	+.081	+.065	+.339	−.072
Aug. 29–30	+3.000	+1.000	+2.300	+.483	+.125	+.042	+.096	+.020
Sept. 6–7	+4.700	+.867	+6.533	+1.700	+.196	+.036	+.272	+.071
Sept. 17–18	+9.900	−2.167	+2.200	+7.600	+.413	−.090	+.092	+.317
Total	+18.566	−5.500	+21.266	+8.517	+.155	−.045	+.177	+.071
Grand total	+10.567	+2.800	+14.301	+14.650	+.044	+.012	+.060	+.061

During the first of the two periods, the apparent rates of photosynthetic activity for the normal tree and normal parts of trees are higher. The rates for the period as a whole are as +0.051 to −0.058, and as +0.069 to −0.067. The differences in total net production of photosynthate are wide, being +6.133 gm. for tree 6 as compared with −6.965 for tree 5, and +8.300 gm. for normal limbs of trees 1 and 3 as compared with −7.999 for the barren portions. The change, of the nature of a reversal, for the second period is worthy of note. The barren tree (5) and the barren parts of the mixed trees (1 and 3) have higher rates and sustain these to the end, which results in much greater amounts of photosynthate. (Note the second line of totals and rates.)

Table 1 (bottom line) gives the totals and rates for the two periods combined, or for the entire season. The values for the barren and normal trees are practically identical, but the values for the barren parts of trees 1 and 3 are very much higher than those for the fruitful limbs. Whether or not this difference, which does not appear in the comparison of the wholly normal and wholly barren trees, is peculiar to trees that are mixtures, through partial reversion, of barrenness and fruitfulness, is a question worthy of interest and attention.

It may be inferred, at least tentatively, that barren mutants in the Montmorency cherry are, as compared with the normal form, photosynthetically deficient during the period of fruit-bud initiation, differ-

entiation, and early deve'opment, but not so with respect to the later part of the season or the season as a whole.

VEGETATIVE GROWTH MEASUREMENTS

In a study of the vegetative development of the trees made in 1937, increase in the diameter of the twig, on the wood of the previous year, was taken as the criterion for growth. The twigs to be measured were chosen over the tree, or part of the tree, on May 5. These were permanently marked on this date of selection and first measurement (by means of a caliper), and thereafter remeasured at designated times during the remainder of the season.

Certain branches on tree 6, and also on the normal fruiting branches of tree 3, were deflorated immediately after flowering. This relieved the trees of the burden of setting and developing fruit and was expected to cause their approach to the barren forms in the magnitude of vegetative growth as determined by increase in diameter of twigs. The results are given in table 2.

TABLE 2.—*Growth in diameter of twigs on barren, fruitful, and deflorated trees, 1937*

Tree No.	Location of twigs on branches	Twigs	Fruit	Average diameter of twigs on—										Gain
				May 5	May 22	June 14	July 7	July 22	Aug. 2	Aug. 12	Aug. 23	Sept. 1	Sept. 13	
		No.	*Lb.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Pct.*
1	Barren	63	.....	3.6	3.6	4.3	5.0	5.2	5.3	5.4	5.4	5.5	5.5	53
	Fruitful	62	36	2.9	2.9	3.3	3.7	3.9	4.0	4.0	4.2	4.2	4.2	45
3	Barren	88	.....	3.1	3.3	3.9	4.4	4.9	5.0	5.1	5.2	5.3	5.3	71
	Deflorated	55	.....	2.8	2.9	3.3	3.8	4.1	4.2	4.3	4.4	4.5	4.5	61
	Fruitful	55	12	2.7	2.9	3.2	3.5	3.8	3.8	3.9	4.0	4.1	4.1	52
5	Barren	85	.....	3.3	3.5	4.3	5.0	5.4	5.5	5.6	5.8	5.9	5.9	79
6	Fruitful	51	[1] 20	3.0	3.2	3.5	3.8	4.1	4.2	4.2	4.3	4.5	4.5	50
	Deflorated	66	.....	3.0	3.1	3.7	4.3	4.7	4.8	4.8	5.0	5.1	5.3	77

[1] For experimental branches; for entire tree, 88 pounds.

The data in table 2 afford no surprises. The relative gains in purely vegetative growth of the barren forms for the entire season exceeded those of the normal by significant differences. The latter, of course, sustained the additional outlay of manufactured materials required for the processes of blooming, fruit development, and fruit-bud formation. Where this drain was checked by defloration (in trees 3 and 6), and this supply released from the usual channel of fruit development, the twigs on the deflorated branches responded by making gains similar to those for the mutationally barren branches. And, furthermore, these branches adhered to the normal course of behavior by forming fruit buds for the year to follow. Their close approach to the barren form, when deflorated, suggests that most of the restriction on vegetative growth is imposed by the fruit-development phase of the reproductive process; blooming and fruit-bud formation seem to account for only a relatively minor part of it.

DISCUSSION AND SUMMARY

The results obtained justify certain general conclusions. Barren mutants in the Montmorency cherry, whether as whole trees or as parts of trees, are not, on the whole, lower in photosynthetic activity than normal trees. Their total, seasonal supply of organic materials is not too small for fruit-bud formation, with subsequent blooming and fruit development. This nutrient supply, however, goes directly and completely into vegetative growth, whereas in the normal form it is partly diverted to the requirements of the several phases of sexual reproduction. And this, it may be said, is the basic peculiarity of the genetic condition within the mutant. Affected and fixed in nature by a means known as mutation, it is artificially producible, to some extent, in the normal form by other means, such as defloration.

The results of the investigation, though far from conclusive, suggest an answer to the questions why, specifically, does the mutant fail to form fruit buds, and why at the critical time for their initiation, does its entire food supply continue to go into vegetative channels? The data seem to show that this is due to a lowered rate of photosynthetic activity which precludes an excess of nutrient materials beyond that required by and taken for the continuation of vegetative growth. In comparisons of the mutant and normal forms the mutant was distinctly below the normal in photosynthetic production just prior to and during the period of detectable fruit-bud differentiation. Moreover, this appears to have been a particular depression in the rate of photosynthesis, for it was clearly below that which obtained after the close of this period and also below that for the entire season. Additional support for this explanation is provided by the analysis of the spurs. Fruit-bud differentiation is not dependent solely on the presence of an excess of organic materials; the location of this excess is also important. Apparently it must occur in the tissues of the spurs as the principal fruit-bud-forming structures of the tree. In the mutant forms these structures were lower than in the normal form in free reducing substances and starch, from early in June—at least a month before observable fruit-bud differentiation—until the end of fruit-bud formation. During this period the mutant's rate of vegetative growth, as shown by table 2, was at its highest, and also at its peak of excess over that of the normal form. Hence, there appears to have been abundant and ample synthesis of organic materials. In the mutant, however, this supply goes promptly into vegetative growth, with relatively slight deposition and accumulation in the spurs. This tendency of the mutant, prohibitive as it is of incipient preparation for fruit-bud initiation, continues throughout the period of fruit-bud differentiation.

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